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(54) METHODS OF CREATING DWARF PHENOTYPES IN PLANTS

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(57) ABSTRACT

The invention is directed to the application of gene sequences which cause a dwarf phenotype in plants to the fields of forestry plants, ornamental horticultural plants, medicinal plants, and Nicotiana plants which are used for purposes other than for traditional tobacco products. The invention provides cDNAs identified by the polynucleotide sequences SEQ ID NO: 1-122 that may be used to create transfected or transgenic plants exhibiting a dwarf phenotype. The invention also provides methods of creating a transfected or transgenic plant exhibiting a dwarf phenotype by expressing in the plant DNA or mRNA identified by the sequences SEQ ID NO:1-122.

<u>Column:</u> t hickness	Chrom	pack CPSil 80	CB, 50 m x 0.32	mm i.d. with ().25 micron film
<u>Oven</u>					
Euilibration Time		e			2. O minutos
Initial Temperatu	re: 50°C		Initial Time	:	3.0 minutes
Ramps:	<u>Rate (°C/</u>	min) <u>Final</u>	<u>Temp(°C)</u> <u>Fi</u>	nal Time (min	ر د
#1	30	25	50	0.00	
#2	25	34	10	5.50	
#3	0				
Front and Back	<u>Inlet</u>				
Mode:	Split		Initial Tem		250°C
Pressure:	15 psig		Split Ratio:		5:1
Split Flow:	23.7 ml	_/min	Total Flow:		35.2 mL/min
Gas Saver:	Off		Gas:		Hydrogen
Mode:	Rampe	l pressure			
Initial Pressure:	•		Initial Time	: 0.0 min	ites
Rate (psig/1	min) <u>Final Pre</u>	s. (psig) <u>F</u>	inal Time (min.)		
5	40		10.0		
10	50		3.0		
Post Pre					
Nominal Initial F			Average ve	locity:	68 cm/sec
Detector (Flame	e Ionization Dete	ctor; FID)		_	
Temperature:	350°C		Hydrogen H	Flow:	40.0 mL/min
Air Flow:	400 mI		Mode:	m	Constant column flow
Makeup Flow:	25.0 m	L/min	Makeup Ga	as Type:	Nitrogen
Electrometer:	On		Lit offset:	D	2.0
Flame:	On		Signal Data	i Kate:	10 Hz
Zero:	0		Range:		0
Fast Peaks:	Off		Attenuation	1:	0
APEX Injector					
Injector Mode Pr	rogram	-	D. 1		
		Front	Back		
	Mode	<u>Minutes</u>	<u>Minutes</u>		
Initial	GC Split	0.00	0.00		
1	Splitless	0.20	1.25		
2	Prosep Split	4.00	6.00		
3	GC Split	6.00	8.00		
Precolumn Temp	perature Program		T2	Deale	
		m (C)	Front	Back	n
	Rate (C/min)	Target (C)	Minutes	<u>Minute</u>	2
		50	0.20	1.25	
1	300	400	10.00	10.00	

Figure 1a. GC/FID Conditions for the Analysis of Tobacco Metabolites in Fraction 1

Liners:	Restek split/split silanized glass w	less single-tape ool.	r liner 4 1	nm i.d (bo	orosilicate) without
<u>Column:</u>	DB23 from J & V	W, 15 m x 0.25	mm i.d. v	vith 0.15 r	nicron film thickness
<u>Oven</u> Equilibration Time:	0.1 minute				
Initial Temperature:	70°C	Initial	Time:	2.0 minu	ites
Ramps:	Rate (°C/min) F	inal Temp(°C)		<u>fime (min.</u>	.)
#1	25	170		0.00	
#2	10	220		1.00	
Dual injection mode:	Start program with	front injection.			
Front Inlet					
Mode:	Splitless	Tempe	erature:	230°C	
Pressure:	12.9 psi	Spliles	s: NA		
Split vent	time: 1.00 min	Flow:	35 mL/m	in	
Gas Saver:	On (5 minutes)	Flow:	20 mL/m	in	
Gas:	Helium				
Mode:	2 ml/min constan	nt flow			
Total Flow:	24.5 mL/min		ge velocit	y: 53 cm/s	sec
Back Inlet					
Mode:	Splitless	Temp	erature:	230°C	
	12.9 psi	Spliles			
Pressure:	time: 2.00 min		35 mL/m	in	
Split vent Gas Saver:	On (5 minutes)		20 mL/m		
	Helium	110011	20 1112,112		
Gas:	2 ml/min constar	ot flow			
Mode:	24.5 mL/min		ge velocit	ty: 53 cm/s	sec
Total Flow:	24.5 mil/min	Avera	ge veloen	.y. 00 0110	
<u>Detectors (Flame Ioni</u>		<u>D)</u>			40.0 mL/min
Temperature:	240°C	-	gen Flow	:	Constant column flow
Air Flow:	400 mL/min	Mode			
Makeup Flow:	25.0 mL/min	Make	up Gas Ty	/pe:	Nitrogen
Electrometer:	On	Lit of			2.0
Flame:	On		l Data Ra	te:	10 Hz
Zero:	0	Range			0
Fast Peaks:	Off	Atten	uation:		0
Fraction 3					

Figure 1b. GC/FID Conditions for the Analysis of Tobacco Metabolites in Fraction 2

<u>Column:</u> thickness	Chrompack	CPSil 8CB, 50 m	x 0.32 mm i.d. v	vith 0.25 micron film
<u>Oven</u> Euilibration Time: Initial Temperature	1 minute : 50°C	Initia	l Time:	2.0 minutes
Ramps: #1 #2 #3	<u>Rate (°C/min)</u> 30 25 0	<u>Final Temp(°C</u> 250 325	Final Time 0.00 3.00	
Front and Back In Mode: Pressure: Split Flow: Gas Saver: Mode: Initial Pressure: 1	Split 15 psig 23.7 mL/min Off Ramped pre	n Tota Gas: ssure		290°C 5:1 35.2 mL/min Hydrogen
Rate (psig/mi	-			
5 10	40 50	10.0 3.0		
Post Press	ure: 15 psig			
Nominal Initial Flo	w: 4.7 mL/min	Ave	age velocity:	68 cm/sec
Detector (Flame Io Temperature: Air Flow: column+makeup Combined Flow:	onization Detector: 350°C 400 mL/min 20 mL/min	n Hyd Mod	rogen Flow: e: eup Flow:	40.0 mL/min Constant 25.0 mL/min
Makeup Gas Type:				
Electrometer: Flame: Zero: Fast Peaks:	On On 0 Off	Sign Ran	ffset: al Data Rate: ge: nuation:	2.0 20 Hz 0 0
APEX Injector				
Inj Volume: Injector Mode Prog	2.5 ul gram Fro	ont Bacl	r.	
Initial C 1 S	ModeMiGC Split0.Splitless0.	<u>nutes Min</u> .00 0.4 .00 0.4	<u>utes</u>)0)0	
		.00 5.0	JU .	
Precolumn Temper	-	From From From From From From From From From		ick i <u>nutes</u>
	1	100 0. 325 10.	87 ().87).00

Figure 1c. GC/FID Conditions for the Analysis of Tobacco Metabolites in Fraction 3

Tigue Id. Der BD Faranceols for the Finally and Fi Teedeter Heart					
Column:Aminoquant Hypersil ODS 5-μm column (200 mm x 2.1 mm)Guard Column:Hypersil ODS 5 μm (20 mm x 2.1 mm)Column Temperature:45 °C					
Agilent 1100 Binary Pum	p Program				
Mobile Phase A:		Buffer pH7.2 containing EDTA (4ug/mL), triethylamine			
	(0.18uL/mL), TH	(0.18uL/mL), THF (0.3%) (v:v)			
Mobile Phase B:	Aqueous Acetate	Buffer pH7.2:methanol:acetonitrile (2:4:4) (v:v:v)			
Pump Program					
Time (min)	% B Fl	ow (mL)			
0.0	0	0.6			
9.5	60	0.6			
10	100	0.6			
10.5	100	1.1			
13.1	100	0.6			
14	0	0.6			
Agilent 1100 Autosampler ProgramStep 1. Draw 5 uL borate bufferStep 2. Draw 1 uL OPA reagentStep 3. Draw 0 uL water (Needle Wash)Step 4. Draw 1 uL sampleStep 5. Mix 7 uL air 5 timesStep 6. Draw 0 uL water (Needle Wash)Step 7. Draw 1 uL FMOC reagentStep 8. Draw 0 uL water (Needle Wash)Step 9. Draw 1 uL borate bufferStep 10. Mix 9 uL air 3 timesStep 11. Inject					
Agilent 1100 Fluorescent Detector					
Time 0.0					
Excitat		340 nm			
Emission:		450 nm			
PMT Gain:		10			
Time 9.2 min	•	266 mm			
Excitat		266 nm 205 nm			
Emissi		305 nm 9			
PMT G	ram:	9			

Figure 1d. LC/FLD Parameters for the Analysis of Tobacco Metabolites in Fraction 4

METHODS OF CREATING DWARF PHENOTYPES IN PLANTS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the priority benefit of provisional U.S. Patent Application Serial No. 60/219,943, filed Jul. 20, 2000, which is hereby incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to nucleic acids and amino acid sequences identified in multiple metabolic pathways that lead to dwarfism and stunting in plants and the use of these sequences to create dwarf varieties of any plant species. Particularly, this invention relates to the use of nucleic acids and amino acid sequences which cause dwarfing in the fields of forestry plants, ornamental horticultural plants, medicinal plants, and Nicotiana plants.

BACKGROUND OF THE INVENTION

[0003] The strategies for increasing the productivity of plants is dependent on rapid discovery of unknown gene sequences and their function through genomics research. These discoveries will provide fundamental information necessary to engineer plants for improved grain yields and resistance to drought, pests, salt, and other extreme environmental conditions. Such advances are critical for a world population expected to double by 2050. Moreover, this information may identify genes and products encoded by genes that are useful for human and animal healthcare such as pharmaceuticals.

[0004] There has been a massive accumulation of expressed sequence tags (ESTs) as a result of recent genome research. Potential use of this sequence information is enormous once gene function is determined. Knowledge of function allows engineering of commercial plants and seeds for forestry, ornamental and horticultural plants, including any plants used to produce pharmaceutical products, and particularly plants of the genus Nicotiana for purposes other than traditional tobacco products.

[0005] Use of these sequences to convey any number of desirable traits to pharmaceutical and fiber crops and thereby increase production and building materials, medicines and chemicals for other uses. For example, gene profiling in cottonwood may lead to an understanding of the types of genes and promoters that act primarily in fiber cells. The novel sequences derived from these profiling studies may be important in genetic engineering of cottonwood fiber for increased strength. In plant breeding, gene profiling coupled to physiological trait analysis can lead to the identification of predictive markers that will be increasingly important in marker assisted breeding programs. Mining the DNA sequence of a particular crop for genes important for yield, quality, health, appearance, color, taste, etc. are applications of obvious importance for crop improvement.

[0006] The Green Revolution crops, introduced in the late 1960s and early 1970s, produce several times as much grain as the traditional varieties they replaced, and they spread rapidly. They enabled India to double its wheat crop in seven years, dramatically increasing food supplies and averting

widely predicted famine. The Green Revolution's leading research achievement was to hasten the perfection of dwarf spring wheat. Though it is conventionally assumed that farmers want a tall, impressive-looking harvest, in fact shrinking wheat and other crops has often proved beneficial. When bred for short stalks, plants expend less energy growing inedible column sections and more growing valuable grain. Stout, short-stalked wheat also neatly supports its kernels, whereas tall-stalked wheat may bend over at maturity, complicating reaping. Nature has favored genes for tall stalks, because in nature plants must compete for access to sunlight. However, in high-yield agriculture, equally shortstalked plants will receive equal sunlight. Researchers are actively seeking dwarf strains of rice and other crops in order to increase agronomic yields. The identification of genes and metabolic pathways that may be modified to create rapidly growing dwarf strains would greatly accelerate this effort. Furthermore, identification of these genes and metabolic pathways in food crops may lead to the development of dwarf strains in other plant types such as forest trees, ornamental species such as ornamental and turfgrass, and plants such as Nicotiana sp. grown as hosts for biopharmaceutical manufacturing.

SUMMARY OF THE INVENTION

[0007] The invention is directed to the application of gene sequences which cause a dwarf phenotype in plants to the fields of forestry plants, ornamental horticultural plants, medicinal plants, and Nicotiana plants which are used for purposes other than for traditional tobacco products.

[0008] The invention provides cDNAs identified by the polynucleotide sequences SEQ ID NO: 1-122 that may be used to create transfected or transgenic plants exhibiting a dwarf phenotype. These cDNAs have been identified by phenotypic screening of the Large Scale Biology's libraries over 8000 cDNAs from Arabidopsis, Nicotiana, Oryza and Papaver constructed in the GENEWARE® vector.

[0009] The invention provides methods of creating a transfected or transgenic plant exhibiting a dwarf phenotype comprising: expressing in the plant a cDNA (or its encoded mRNA) identified by a polynucleotide sequence chosen from the group consisting of SEQ ID NO: 1-122.

[0010] The invention also provides a method of creating a transfected or transgenic plant exhibiting a dwarf phenotype comprising the steps of: (a) providing a viral inoculum capable of infecting a plant comprising the cDNA (or its encoded mRNA) identified by a polynucleotide sequence chosen from the group of SEQ ID NO: 1-122; and (b) applying said viral inoculum to a plant; whereby the plant is infected and the cDNA (or its encoded mRNA) is expressed in the plant.

[0011] The methods of the invention provide for creating a transfected or transgenic plant exhibiting a dwarf phenotype in any plant type. Preferred embodiments of the invention provide methods for creating dwarf plants of ornamental and horticultural plants, medicinal plants or forest trees. A preferred embodiment provides methods for creating dwarf plants of Nicotiana sp. Another preferred embodiment provides methods for creating dwarf turfgrass.

[0012] The invention also provides methods for creating transfected or transgenic plants exhibiting a dwarf pheno-

type for use in biopharmaceutical manufacturing comprising: applying a viral inoculum capable of infecting a plant and comprising the DNA (or its encoded mRNA) identified by a polynucleotide sequence chosen from the group of SEQ. ID NO 1-122 to a plant that expresses a biopharmaceutical, whereby the plant is infected, exhibits a dwarf phenotype, and expresses the biopharmaceutical.

[0013] The invention also provides a transfected or transgenic plant exhibiting a dwarf phenotype made by the method comprising expressing in the plant a cDNA(or its encoded mRNA) identified by a polynucleotide sequence chosen from the group consisting of SEQ ID NO: 1-122. The invention provides for transfected or transgenic plants made by the use of this method with any plant type. Preferred embodiments are transfected or transgenic plants of ornamental and horticultural plants, medicinal plants or forest trees. Preferred embodiments include transfected or transgenic plants of Nicotiana sp and dwarf turfgrass.

[0014] The invention also provides methods of producing multiple crops of the transfected or transgenic plants expressing a cDNA(or its encoded mRNA) identified by a polynucleotide sequence chosen from the group consisting of SEQ ID NO: 1-122 and exhibiting a dwarf phenotype comprising the steps of: (a) planting a reproductive unit of the transfected or transgenic plant; (b) growing the planted reproductive unit under natural light conditions; (c) harvesting the plant; and (d) repeating steps (a) through (c) at least once in the year.

[0015] The invention provides a method of constructing and characterizing a normalized cDNA library in a viral vector. The invention further provides a method of constructing and characterizing of a normalized whole plant cDNA library in viral vectors.

[0016] The invention identifies cDNAs corresponding to genes in the trans-ketolase and carbohydrate metabolic pathways as useful for creating transfected or transgenic plants exhibiting a dwarf phenotype.

[0017] The invention also provides method of manufacturing a biopharmaceutical comprising:

DESCRIPTION OF THE INVENTION

[0018] Before the present proteins, nucleotide sequences, and methods are described, it should be noted that this invention is not limited to the particular methodology, protocols, plants, cell lines, vectors, and reagents described herein as these may vary. It should also be understood that the terminology used herein is for the purpose of describing particular aspects of the invention, and is not intended to limit its scope which will be limited only by the appended claims.

[0019] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the "antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

[0020] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as com-

monly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

- [0021] "Acylate" as used herein, refers to the introduction of an acyl group into into a molecule, i.e. acylation
- [0022] "Adjacent" as used herein, refers to a position in a nucleotide sequence proximate to and 5' or 3' to a defined sequence. Generally, adjacent means within 2 or 3 nucleotides of the site of reference.
- **[0023]** "Agonist", as used herein, refers to a molecule which, when bound to a gene product of interest, increases the biological or immunological activity of that gene product. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to a gene product of interest.
- **[0024]** "Alterations" in a polynucleotide sequence, as used herein, comprise any deletions, insertions, and point mutations in the polynucleotide sequence. Included within this definition are alterations to any genomic DNA sequence corresponding to the polynucleotide sequence.
- [0025] "Amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules. "Amino acid sequence" and like terms, such as "polypeptide" or "protein" as recited herein are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.
- [0026] "Amplification" as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C. W. and G. S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y.).
- [0027] "Antibody" refers to intact molecules as well as fragments thereof which are capable of specific binding to the epitopic determinant. Antibodies that bind a polypeptide of interest can be prepared using intact polypeptides or fragments as the immunizing antigen. These antigens may be conjugated to a carrier protein, if desired.
- **[0028]** "Antigenic determinant," "determinant group," or "epitope of an antigenic macromolecule" as used herein, refers to any region of the macromolecule with the ability or potential to elicit, and combine with, specific antibody. Determinants exposed on the surface of the macromolecule are likely to be immunodomi-

nant, i.e. more immunogenic than other (imunorecessive) determinants which are less exposed, while some (e.g. those within the molecule) are non-immunogenic (immunosilent). As used herein, antigenic determinant refers to that portion of a molecule that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

- [0029] "Antisense", as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense" or "(-) sense" is used in reference to the nucleic acid strand that is complementary to the "sense" or "(+) sense" strand. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene of interest in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, the transcript of this strand may hybridize to natural sequences to block either their further transcription or translation. In this manner, mutant phenotypes may be generated.
- [0030] "Anti-Sense Inhibition" as used herein, refers to a type of gene regulation based on cytoplasmic, nuclear or organelle inhibition of gene expression due to the presence in a cell of an RNA molecule complementary to at least a portion of the mRNA being translated. It is specifically contemplated that DNA molecules may be from either an RNA virus or mRNA from the host cells genome or from a DNA virus.
- [0031] "Antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to a gene product of interest, decreases the biological or immunological activity of that gene product of interest. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to the gene product of interest.
- **[0032]** "Biologically active", as used herein, refers to a molecule having the structural, regulatory, or biochemical functions of a naturally occurring molecule.
- [0033] "Cell Culture" as used herein, refers to a proliferating mass of cells which may be in either an undifferentiated or differentiated state, growing contiguously or non-contiguously.
- **[0034]** "Chimeric plasmid" as used herein, refers to any recombinant plasmid formed (by cloning techniques) from nucleic acids derived from organisms which do not normally exchange genetic information (e.g. *Escherichia coli* and *Saccharomyces cerevisiae*).
- [0035] "Chimeric Sequence" or "Chimeric Gene" as used herein, refers to a nucleotide sequence derived from at least two heterologous parts. The sequence may comprise DNA or RNA.

- [0036] "Coding Sequence" as used herein, refers to a nucleic acid sequence which, when transcribed and translated, results in the formation of a cellular polypeptide or a ribonucleotide sequence which, when translated, results in the formation of a cellular polypeptide.
- [0037] "Common Embryological Basis" as used herein, is intended to include all tissues which are derived from the same germinal layer, specifically the ectoderm layer, which forms during the gastrulation stage of embryogenesis. Such tissues include, but are not limited to, brain, epithelium, adrenal medulla, spinal chord, retina, ganglia and the like.
- [0038] "Compatible" as used herein, refers to the capability of operating with other components of a system. A vector or plant viral nucleic acid which is compatible with a host is one which is capable of replicating in that host. A coat protein which is compatible with a viral nucleotide sequence is one capable of encapsidating that viral sequence.
- [0039] "Complementary" or "Complementarity", as used herein, refer to the Watson-Crick base-pairing of two nucleic acid sequences. For example, for the sequence 5'-AGT-3' binds to the complementary sequence 3'-TCA-5'. Complementarity between two nucleic acid sequences may be "partial", in which only some of the bases bind to their complement, or it may be complete as when every base in the sequence binds to it complementary base. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.
- **[0040]** "Complementation analysis" as used herein, refers to observing the changes produced in an organism when a nucleic acid sequence is introduced into that organism after a selected gene has been deleted or mutated so that it no longer functions fully in its normal role. A complementary gene to the deleted or mutated gene can restore the genetic phenotype of the selected gene.
- **[0041]** "Constitutive expression" as used herein refers to gene expression which features substantially constant or regularly cyclical gene transcription. Generally, genes which are constitutively expressed are substantially free of induction from an external stimulus.
- **[0042]** "Correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to and indicative of the presence of an mRNA encoding a polypeptide in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.
- [0043] "Deletion", as used herein, refers to a change made in either an amino acid or nucleotide sequence resulting in the absence one or more amino acids or nucleotides, respectively.
- [0044] "Differentiated cell" as used herein refers to a cell which has substantially matured to perform one or more biochemical or physiological functions.

- [0045] "Dwarf Plant" as used herein, refers to a plant that is much below the height or size of its kind or related species.
- [0046] "Encapsidation" as used herein, refers to the process during virion assembly in which nucleic acid becomes incorporated in the viral capsid or in a head/ capsid precursor (e.g. in certain bacteriophages).
- [0047] "Exon" as used herein, refers to a polynucleotide sequence in a nucleic acid that codes information for protein synthesis and that is copied and spliced together with other such sequences to form messenger RNA.
- **[0048]** "Expression" as used herein is meant to incorporate one or more of transcription, reverse transcription and translation.
- **[0049]** "Expressed sequence tag (EST)" as used herein refers to relatively short single-pass DNA sequences obtained from one or more ends of cDNA clones and RNA derived therefrom. They may be present in either the 5' or the 3' orientation. ESTs have been shown useful for identifying particular genes.
- [0050] "Foreign gene" as used herein, refers to any sequence that is not native to the virus.
- **[0051]** "Fusion protein" as used herein, refers to a protein containing amino acid sequences from each of two distinct proteins; it is formed by the expression of a recombinant gene in which two coding sequences have been joined together such that their reading frames are in phase. Hybrid genes of this type may be constructed in vitro in order to label the product of a particular gene with a protein which can be more readily assayed (e.g. a gene fused with lacZ in *E. coli* to obtain a fusion protein with β -galactosidase activity). Alternatively, a protein may be linked to a signal peptide to allow its secretion by the cell. The products of certain viral oncogenes are fusion proteins.
- [0052] "Gene" as used herein, refers to a discrete nucleic acid sequence responsible for a discrete cellular product and/or performing one or more intercellular or intracellular functions. The term "gene", as used herein, refers not only to the nucleotide sequence encoding a specific protein, but also to any adjacent 5' and 3' non-coding nucleotide sequence involved in the regulation of expression of the protein encoded by the gene of interest. These non-coding sequences include terminator sequences, promoter sequences, upstream activator sequences, regulatory protein binding sequences, and the like. These non-coding sequence gene regions may be readily identified by comparison with previously identified eukaryotic non-coding sequence gene regions. Furthermore, the person of average skill in the art of molecular biology is able to identify the nucleotide sequences forming the noncoding regions of a gene using well-known techniques such as a site-directed mutagenesis, sequential deletion, promoter probe vectors, and the like.
- **[0053]** "Growth cycle" as used herein is meant to include the replication of a nucleus, an organelle, a cell, or an organism.
- [0054] "Half-life" as used herein, refers to the time required for half of something to undergo a process

(e.g. the time required for half the amount of a substance, such as a drug or radioactive tracer, in or introduced into a living system or ecosystem to be eliminated or disintegrated by natural processes.

- [0055] "Heterologous" as used herein, refers to the association of a molecular or genetic element associated with a distinctly different type of molecular or genetic element.
- **[0056]** "Host" as used herein, refers to a cell, tissue or organism capable of replicating a vector or plant viral nucleic acid and which is capable of being infected by a virus containing the viral vector or plant viral nucleic acid. This term is intended to include procaryotic and eukaryotic cells, organs, tissues or organisms, where appropriate.
- [0057] "Homology" as used herein, refers to the degree of similarity between two or more nucleotide or aminoacid sequences. Homology may be partial or complete.
- **[0058]** "Hybridization", as used herein, refers to any process by which a strand of nucleic acid binds with a complementary or partially complementary strand through base pairing.
- [0059] "Hybridization complex", as used herein, refers to a complex formed between nucleic acid strands by virtue of hydrogen bonding, stacking or other noncovalent interactions between bases. A hybridization complex may be formed in solution or between nucleic acid sequences present in solution and nucleic acid sequences immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed for in situ hybridization).
- **[0060]** "Immunologically active" refers to the capability of a natural, recombinant, or synthetic gene product of interest, or any oligopeptide thereof, to bind with specific antibodies and induce a specific immune response in appropriate animals or cells.
- [0061] "Induction" and the terms "induce", "induction" and "inducible" as used herein, refer generally to a gene and a promoter operably linked thereto which is in some manner dependent upon an external stimulus, such as a molecule, in order to actively transcribed and/or translate the gene.
- [0062] "Infection" as used herein refers to the ability of a virus to transfer its nucleic acid to a host or introduce a viral nucleic acid into a host, wherein the viral nucleic acid is replicated, viral proteins are synthesized, and new viral particles assembled. In this context, the terms "transmissible" and "infective" are used interchangeably herein. The term is also meant to include the ability of a selected nucleic acid sequence to integrate into a genome, chromosome or gene of a target organism.
- [0063] "Insertion" or "Addition", as used herein, refers to the replacement or addition of one or more nucleotides or amino acids, to a nucleotide or amino acid sequence, respectively.
- [0064] "In cis" as used herein, indicates that two sequences are positioned on the same strand of RNA or DNA.

- [0065] "In trans" as used herein, indicates that two sequences are positioned on different strands of RNA or DNA.
- **[0066]** "Intron" as used herein refers to a polynucleotide sequence in a nucleic acid that does not code information for protein synthesis and is removed before translation of messenger RNA.
- [0067] "Isolated" as used herein refers to a polypeptide, polynucleotide molecules separated not only from other peptides, DNAs, or RNAs, respectively, that are present in the natural source of the macromolecule but also from other macromolecules and preferably refers to a macromolecule found in the presence of (if anything) only a solvent, buffer, ion or other component normally present in a solution of the same. "Isolated" and "purified" do not encompass either natural materials in their native state or natural materials that have been separated into components (e.g., in an acrylamide gel) but not obtained either as pure substances or as solutions.
- **[0068]** "Kinase" as used herein, refers to an enzyme (e.g. hexokinase and pyruvate kinase) which catalyzes the transfer of a phosphate group from one substrate (commonly ATP) to another.
- [0069] "Marker" or "Genetic Marker" as used herein, refers to a genetic locus which is associated with a particular, usually readily detectable, genotype or phenotypic characteristic (e.g., an antibiotic resistance gene).
- [0070] "Metabolome" as used herein, indicates the complement of relatively low molecular weight molecules that is present in a plant, plant part, or plant sample, or in a suspension or extract thereof. Examples of such molecules include, but are not limited to: acids and related compounds; mono-, di-, and tri-carboxylic acids (saturated, unsaturated, aliphatic and cyclic, aryl, alkaryl); aldo-acids, keto-acids; lactone forms; gibberellins; abscisic acid; alcohols, polyols, derivatives, and related compounds; ethyl alcohol, benzyl alcohol, menthanol; propylene glycol, glycerol, phytol; inositol, furfuryl alcohol, menthol; aldehydes, ketones, quinones, derivatives, and related compounds; acetaldehyde, butyraldehyde, benzaldehyde, acrolein, furfural, glyoxal; acetone, butanone; anthraquinone; carbohydrates; mono-, di-, tri-saccharides; alkaloids, amines, and other bases; pyridines (including nicotinic acid, nicotinamide); pyrimidines (including cytidine, thymine); purines (including guanine, adenine, xanthines/hypoxanthines, kinetin); pyrroles; quinolines (including isoquinolines); morphinans, tropanes, cinchonans; nucleotides, oligonucleotides, derivatives, and related compounds; guanosine, cytosine, adenosine, thymidine, inosine; amino acids, oligopeptides, derivatives, and related compounds; esters; phenols and related compounds; heterocyclic compounds and derivatives; pyrroles, tetrapyrroles (corrinoids and porphines/porphyrins, w/w/o metal-ion); flavonoids; indoles; lipids (including fatty acids and triglycerides), derivatives, and related compounds; carotenoids, phytoene; and sterols, isoprenoids including terpenes.
- **[0071]** "Modulate" as used herein, refers to a change or an alteration in the biological activity of a gene product

of interest. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional or immunological properties of the gene product of interest.

- **[0072]** "Movement protein" as used herein refers to a noncapsid protein required for cell to cell movement of replicons or viruses in plants.
- [0073] "Multigene family" as used herein refers to a set of genes descended by duplication and variation from some ancestral gene. Such genes may be clustered together on the same chromosome or dispersed on different chromosomes. Examples of multigene families include those which encode the histones, hemoglobins, immunoglobulins, histocompatibility antigens, actins, tubulins, keratins, collagens, heat shock proteins, salivary glue proteins, chorion proteins, cuticle proteins, yolk proteins, and phaseolins.
- [0074] "Non-Native" as used herein refers to any RNA or DNA sequence that does not normally occur in the cell or organism in which it is placed. Examples include recombinant plant viral nucleic acids and genes or ESTs contained therein. That is, a RNA or DNA sequence may be non-native with respect to a viral nucleic acid. Such a RNA or DNA sequence would not naturally occur in the viral nucleic acid. Also, a RNA or DNA sequence may be non-native with repect to a host organism. That is, such a RNA or DNA sequence would not naturally occur in the host organism. Conversely, the term non-native does not imply that a RNA or DNA sequence must be non-native with respect to both a viral nucleic acid and a host organism concurrently. The present invention specifically contemplates placing a RNA or DNA sequence which is native to a host organism into a viral nucleic acid in which it is nonnative.
- [0075] "Nucleic acid sequence" as used herein refers to a polymer of nucleotides in which the 3' position of one nucleotide sugar is linked to the 5' position of the next by a phosphodiester bridge. In a linear nucleic acid strand, one end typically has a free 5' phosphate group, the other a free 3' hydroxyl group. Nucleic acid sequences may be used herein to refer to oligonucleotides, or polynucleotides, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. The term is intended to encompass all nucleic acids whether naturally occurring in a particular cell or organism or non-naturally occurring in a particular cell or organism.
- **[0076]** "Operably Linked" refers to a juxtaposition of components, particularly nucleotide sequences, such that the normal function of the components can be performed. Thus, a coding sequence that is operably linked to regulatory sequences refers to a configuration of nucleotide sequences wherein the coding sequences can be expressed under the regulatory control i.e., transcriptional and/or translational control, of the regulatory sequences.
- [0077] "Organism" and "host organism" as used herein is specifically intended to include animals (including humans), plants, viruses, fungi, and bacteria.

- **[0078]** "Origin of Assembly" as used herein, refers to a sequence where self-assembly of the viral RNA and the viral capsid protein initiates to form virions.
- **[0079]** "Outlier Peak" as used herein, indicates a peak of a chromatogram of a test sample, or the relative or absolute detected response data, or amount or concentration data thereof. An outlier peak: 1) may have a significantly different peak height or area as compared to a like chromatogram of a control sample; or 2) be an additional or missing peak as compared to a like chromatogram of a control sample.
- **[0080]** "Phenotype" or "Phenotypic Trait(s)" as used herein, refers to an observable property or set of properties resulting from the expression or suppression of a gene or genes.
- [0081] "Plant" as used herein refers to any plant and progeny thereof, and to parts of plants including parts of plants, including seed, cuttings, tubers, fruit, flowers, branches, leaves, plant cells and other parts of any tree or other plant used in forestry, ornamental horticultural plants, medicinal plants including any plants used to produce pharmaceutical products, and plants of the genus Nicotiana which are used for purposes other than for traditional tobacco products.
- **[0082]** "Plant Cell" as used herein, refers to the structural and physiological unit of plants, consisting of a protoplast and the cell wall.
- **[0083]** "Plant Organ" as used herein, refers to a distinct and visibly differentiated part of a plant, such as root, stem, leaf or embryo.
- [0084] "Plant Tissue" as used herein, refers to any tissue of a plant in planta or in culture. This term is intended to include a whole plant, plant cell, plant organ, protoplast, cell culture, or any group of plant cells organized into a structural and functional unit.
- [0085] "Portion" as used herein, with regard to a protein (i.e. "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid.
- **[0086]** "Positive-sense inhibition" as used herein refers to a type of gene regulation based on cytoplasmic inhibition of gene expression due to the presence in a cell of an RNA molecule substantially homologous to at least a portion of the mRNA being translated.
- **[0087]** "Production Cell" as used herein, refers to a cell, tissue or organism capable of replicating a vector or a viral vector, but which is not necessarily a host to the virus. This term is intended to include prokaryotic and eukaryotic cells, organs, tissues or organisms, such as bacteria, yeast, fungus and plant tissue.
- **[0088]** "Promoter" as used herein, refers to the 5'-flanking, non-coding sequence substantially adjacent a coding sequence which is involved in the initiation of transcription of the coding sequence.
- **[0089]** "Protoplast" as used herein, refers to an isolated plant cell without cell walls, having the potency for regeneration into cell culture or a whole plant.

- [0090] "Purified" as used herein when referring to a peptide or nucleotide sequence, indicates that the molecule is present in the substantial absence of other biological macromolecular, e.g., polypeptides, polynucleic acids, and the like of the same type. The term "purified" as used herein preferably means at least 95% by weight, more preferably at least 99.8% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 can be present). The term "purified" as used herein preferably has the same numerical limits as "purified" immediately above.
- **[0091]** "Substantially purified" as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.
- [0092] "Recombinant Plant Viral Nucleic Acid" as used herein, refers to a plant viral nucleic acid which has been modified to contain non-native nucleic acid sequences. These non-native nucleic acid sequences may be from any organism or purely synthetic, however, they may also include nucleic acid sequences naturally occurring in the organism into which the recombinant plant viral nucleic acid is to be introduced.
- [0093] "Recombinant Plant Virus" as used herein, refers to a plant virus containing a recombinant plant viral nucleic acid.
- [0094] "Regulatory region" or "Regulatory sequence" as used herein in reference to a specific gene refers to the non-coding nucleotide sequences within that gene that are necessary or sufficient to provide for the regulated expression of the coding region of a gene. Thus the term regulatory region includes promoter sequences, regulatory protein binding sites, upstream activator sequences, and the like. Specific nucleotides within a regulatory region may serve multiple functions. For example, a specific nucleotide may be part of a promoter and participate in the binding of a transcriptional activator protein.
- [0095] "Replication origin" as used herein, refers to the minimal terminal sequences in linear viruses that are necessary for viral replication.
- [0096] "Replicon" as used herein, refers to an arrangement of RNA sequences generated by transcription of a transgene that is integrated into the host DNA that is capable of replication in the presence of a helper virus. A replicon may require sequences in addition to the replication origins for efficient replication and stability.
- [0097] "Sample", as used herein, is used in its broadest sense. A biological sample suspected of containing a nucleic acid or fragments thereof may comprise a tissue, a cell, an extract from cells, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for northern analysis), cDNA (in solution or bound to a solid support), and the like.

- **[0098]** "Silent mutation" as used herein, refers to a mutation which has no apparent effect on the phenotype of the organism.
- **[0099]** "Site-directed mutagenesis" as used herein, refers to the in-vitro induction of mutagenesis at a specific site in a given target nucleic acid molecule.
- **[0100]** "Specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody and a protein or peptide, mean that the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein; in other words, the antibody is recognizing and binding to a specific protein structure rather than to proteins in general.
- [0101] "Stringent conditions", as used herein, is the "stringency" which occurs within a range from about $(T_m-5)^\circ$ C. (i.e. 5 degrees below the melting temperature, T_m , of the probe) to about 20° to 25° C. below T_m . As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences. Also as known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.
- **[0102]** "Subgenomic Promoter" as used herein, refers to a promoter of a subgenomic mRNA of a viral nucleic acid.
- **[0103]** "Substantial Sequence Homology" as used herein, denotes nucleotide sequences that are substantially functionally equivalent to one another. Nucleotide differences between such sequences having substantial sequence homology will be de minimus in affecting function of the gene products or an RNA coded for by such sequence.
- **[0104]** "Substitution", as used herein, refers to a change made in an amino acid of nucleotide sequence which results in the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.
- **[0105]** "Systemic Infection" as used herein denotes infection throughout a substantial part of an organism including mechanisms of spread other than mere direct cell inoculation but rather including transport from one infected cell to additional cells either nearby or distant.
- **[0106]** "Transcription" as used herein, refers to the production of an RNA molecule by RNA polymerase as a complementary copy of a DNA sequence.
- **[0107]** "Transcription termination region" as used herein, refers to the sequence that controls formation of the 3' end of the transcript. Self-cleaving ribozymes and

polyadenylation sequences are examples of transcription termination sequences.

- [0108] "Transformation" as used herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.
- **[0109]** "Transposon" as used herein refers to a nucleotide sequence such as a DNA or RNA sequence which is capable of transferring location or moving within a gene, a chromosome or a genome.
- **[0110]** "Transgenic plant" as used herein refers to a plant which contains a foreign nucleotide sequence inserted into either its nuclear genome or organellar genome.
- **[0111]** "Transcription" as used herein refers to the production of an RNA molecule by RNA polymerase as a complementary copy of a DNA sequence or subgenomic mRNA.
- [0112] "Variants" of a gene product of interest, as used herein, refers to a sequence resulting when the gene product is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Variants may also include sequences with amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art.
- **[0113]** "Vector" as used herein, refers to a self-replicating DNA or RNA molecule which transfers a DNA or RNA segment between cells.
- **[0114]** "Virion" as used herein, refers to a particle composed of viral RNA and viral capsid protein.
- **[0115]** "Virus" as used herein, refers to an infectious agent composed of a nucleic acid encapsidated in a protein. A virus may be a mono-, di-, tri- or multipartite virus.

THE INVENTION

[0116] Identification and Analysis of cDNAs

[0117] The invention is based on the discovery of 122 cDNAs, identified by the polynucleotide sequences SEQ ID NO: 1-122, that may be used to create transfected or

TABLE 1-continued

transgenic plants exhibiting a dwarf phenotype. Table 1 lists the source organism for all 122 cDNAs of the invention (as identified by its SEQ ID NO).

TABLE 1

1Nicotiana benthamianaA2Nicotiana benthamianaA3Arabidopsis thalianaS4Arabidopsis thalianaS5Arabidopsis thalianaS6Arabidopsis thalianaS7Arabidopsis thalianaS8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA14Arabidopsis thalianaA	
2Nicotiana benthamianaA3Arabidopsis thalianaS4Arabidopsis thalianaS5Arabidopsis thalianaS6Arabidopsis thalianaS7Arabidopsis thalianaS8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
4Arabidopsis thalianaS5Arabidopsis thalianaS6Arabidopsis thalianaS7Arabidopsis thalianaS8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
5Arabidopsis thalianaS6Arabidopsis thalianaS7Arabidopsis thalianaS8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
6Arabidopsis thalianaS7Arabidopsis thalianaS8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
7Arabidopsis thalianaS8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
8Arabidopsis thalianaA9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
9Arabidopsis thalianaA10Arabidopsis thalianaA11Arabidopsis thalianaA12Arabidopsis thalianaA13Arabidopsis thalianaA	
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15 Arabidopsis thaliana A	
16 Arabidopsis thaliana A	
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36 Arabidopsis thaliana A	
37 Arabidopsis thaliana A	
38 Arabidopsis thaliana A	
39 Arabidopsis thaliana A	
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41 Arabidopsis thaliana A 42 Arabidopsis thaliana A	
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46 Arabidopsis thaliana A	
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48Arabidopsis thalianaA49Arabidopsis thalianaA	
50 Arabidopsis thaliana A	
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55 Arabidopsis thaliana A 56 Arabidopsis thaliana A	
56 Arabidopsis thaliana A 57 Arabidopsis thaliana A	
58 Arabidopsis thaliana A	
59 Arabidopsis thaliana A	
60 Arabidopsis thaliana A	
61 Arabidopsis thaliana A	
62 Arabidopsis thaliana A	
63 Arabidopsis thaliana A 64 Arabidopsis thaliana A	
65 Arabidopsis thaliana A	
66 Arabidopsis thaliana A	
67 Arabidopsis thaliana A	
68 Arabidopsis thaliana A	

SEQ ID NO.	Source	Sense or Antisense Configuration
69	Arabidopsis thaliana	А
70	Arabidopsis thaliana	A
71	Arabidopsis thaliana	A
72	Arabidopsis thaliana	A
73	Arabidopsis thaliana	A
74	Arabidopsis thaliana	A
75	Arabidopsis thaliana	A
76	Arabidopsis thaliana	A
77	Arabidopsis thaliana	A
78	Arabidopsis thaliana	А
79	Arabidopsis thaliana	A
80	Arabidopsis thaliana	A
81	Arabidopsis thaliana	А
82	Arabidopsis thaliana	A
83	Arabidopsis thaliana	А
84	Arabidopsis thaliana	Â
85	Arabidopsis thaliana	A
86	Arabidopsis thaliana	A
87	Arabidopsis thaliana	А
88	Arabidopsis thaliana	А
89	Arabidopsis thaliana	Ā
90	Arabidopsis thaliana	Ā
91	Arabidopsis thaliana	А
92	Arabidopsis thaliana	А
93	Arabidopsis thaliana	A
94	Arabidopsis thaliana	Ā
95	Arabidopsis thaliana	A
96	Arabidopsis thaliana	A
97	Arabidopsis thaliana	S
98	Arabidopsis thaliana	Ā
99	Arabidopsis thaliana	n.d.
100	Arabidopsis thaliana	n.d.
101	Arabidopsis thaliana	n.d.
102	Arabidopsis thaliana	n.d.
103	Arabidopsis thaliana	n.d.
104	Arabidopsis thaliana	n.d.
105	Arabidopsis thaliana	n.d.
106	Arabidopsis thaliana	n.d.
107	Arabidopsis thaliana	n.d.
108	Arabidopsis thaliana	n.d.
109	Arabidopsis thaliana	n.d.
110	Arabidopsis thaliana	n.d.
111	Arabidopsis thaliana	n.d.
112	Arabidopsis thaliana	A
113	Nicotiana benthamiana	A
114	Nicotiana benthamiana	A
115	Nicotiana benthamiana	А
116	'Nicotiana benthamiana	S
117	Oryza japonica	S
118	Oryza japonica	S
119	Oryza indica	s
120	Oryza indica	ŝ
121	Papaver rhoeas	ŝ
122	Oryza japonica	S
	~ ~ 1	

[0118] The 122 cDNAs of the invention were identified by phenotypic screening and bioinformatic analysis of libraries of over 8000 cDNAs from Arabidopsis, Nicotiana, Oryza and Papaver constructed in the GENEWARE® vector. Table 1 lists whether the cDNA insert is in the sense (S) or antisense (A) configuration in the GENEWARE® vector used for the phenotypic screening. The use of the GENEWARE® vector in the field of genomics has been described in PCT WO 99/36516 published Jul. 22, 1999, which is herein incorporated by reference for all purposes. The general phenotypic screening method (described in greater constructing detail below) involves а GENEWARE® viral nucleic acid vector from each clone of a normalized cDNA library of interest. Each GENEWARE® vector is then used to create an infectious viral unit which is applied to the individual plants of interest. Inoculation with GENEWARE® viral nucleic acid vectors results in a high rate of systemic infection of plants. The TMV based viral vector identified as PBSG1057 which has the ablility to transfect plants has been deposited under the Budapest Treaty at the AFCC and is designated ATCC #203981. Infected (and uninfected) plants are grown under identical conditions and an automated visual phenotypic analysis is conducted of each plant. The phenotypic data including descriptive of various parts of each plant is entered into a matrix-style database created using LIMS software. Once in the database, the phenotypic results are linked to the sequence data and bioinformatic analysis associated with each of the GENEWARE® vector (i.e. each cDNA in the library).

[0119] Out of over 8000 *Nicotiana benthamiana* plants infected by the GENEWARE®, 111 were discovered that exhibited a dwarf phenotype. Sequence analysis of these cDNAs (as described in greater detail below) yielded the identifying nucleic acid sequences SEQ. ID. NOS. 1-111. Bioinformatic analysis of these sequences using BLAST and other methods (described in greater detail below) yielded E.C. annotations for a large number of these sequences.

[0120] Further bioinformatic analysis of the 111 polynucleotide sequences identified an additional 34 cDNAs that may also function to cause dwarf phenotype in plants. Pfam analysis (described in greater detail below) of the 111 cDNAs identified SEQ ID NO:95 and 102 as members of the transketolase functional family, and the pfkb carbohydrate kinase family, respectively. Using this information, the 11 additional sequences (identified by SEQ ID NO: 112-122) were discovered in the LSBC GENEWARE® libraries that are either a member of the transketolase having the same metabolic activity as SEQ ID NO. 95, or a member pfkb carbohydrate kinase families having the same metabolic activity as SEQ ID NO. 102.

[0121] Following the identification of plants exhibiting the dwarf phenotype, biochemical analyses of tissue may be carried out in order to ascertain further details of the expressed cDNAs function. Methods including GC/MS analysis and Maldi-TOF analysis of the tissue have been carried out (described in greater detail below) and yield information on the profile of metabolites and proteins present in the infected plant's tissue. The results of these biochemical analyses are linked to the phenotype, sequence, and other bioinformatic data associated with each of the GENEWARE® vector. Using these biochemical analysis methods, and associated data processing techniques, the identification of at least one variation in the metabolome of an infected (versus an uninfected) plant may ascribe a function to the cDNA of interest.

[0122] According to the present invention, the dwarf phenotype may be created in a wide variety of plants or plant cell systems using the cDNAs identified by SEQ ID NO:1-122 and the various transformation methods described. In preferred embodiments, target plants and plant cells for engineering include, but are not limited to, monocotyledonous and dicotyledonous plants, including horticultural and ornamental plants (e.g., the grass and turfgrass species, and flowering plants such as petunia, rose, chrysanthemum),

conifers and pine trees (e.g., pine, fir, spruce species, and including Abies sp., Acer glabrum, Pinus sp., Alnus sp., Arbutus arizonica, Betula occidentalis, Cedrus sp., Cryptomeriajaponica, Cupressus sp., Eucalyptus sp., Ginkgo biloba, Juniperus sp., Libocedrus decurrens, Liriodendron tulipifera, Lithocarpus densiflora, Metasequoia glyptostroboides, P. ponderosa var. scopulorum, Picea sp., Platanus sp., Populus sp., Pseudotsuga sp., Purshia tridentata, Quercus sp., Sequoia sp., Taxus brevifolia, Thuja sp., Torreya californica, Tsuga heterophylla, Umbellularia californica); plants used in phytoremediation (e.g., heavy metal accumulating plants), medicinal plants (e.g. Solanaceae, Atropa belladonna, Duboisia myoporides, Hyoscymus niger, Scopolina atropoides, Solanum tuberosum, Eschscholtzia californica, Berberis stolonifera, Papaver somniferum) and plants used for experimental purposes (e.g., Arabidopsis thaliana, Nicotiana sp.).

[0123] For a more complete listing of medicinal plants see Table 2. Another treatment of medicinal herbs can be found in, "1999 PDR for Herbal Medicines" 2nd edition, editors, Joerg Gruenwald et al., Medical Economics Company, Montvale, N.J., which is herein incorporated by reference for all purposes.

TABLE 2

Medicinal Plant	Medicinal Plant
Abies lasiocarpa	Juglans major
Abies excelsa	Juniperus communis
Abronia wootonii	Juniperus monosperma
Acacia arabica	Juniperus sibirica
Acacia catechu	Kallstroemia grandiflora
Acacia constricta	Kallstroemia spp.
Acacia greggii	Kalmia angustifolia
Acacia senegal	Kalmia latifolia
Acalypha californica	Kalmia microphylla
Acalypha lindheimeri	Kalmia polifolia
Achillea lanulosa	Karwinskia humboldtiana
Achillea millefolium	Krameria grayi
Achlys triphylla	Krameria lanceolata
Aconitum columbianum	Krameria parvifolia
Acorus calamus	Lactuca serriola
Actaea alba	Lamium amplexicaule
Actea rubra	Larrea tridentata
Adiantum capillus-veneris	Ledum glandulosum
Adiantum jordanii	Ledum groenlandicum
Adiantum pedatum	Leonurus cardiaca
Adoxa moschatellina	Leonurus sibirica
Aesculus californica	Lepechinia calycina
Aesculus glabra	Lepidium montanum
Aesculus hippocastanum	Lespedeza violacea
Aesculus pavia	Leucophyllum frutescens
Agastache urticifolia	Levisticum ligusticum
Agave chisoensis	Lewisia rediviva
Agave parryi	Liatris punctata
Agrimonia gryposepala	Liatris squarrosa
Agrimonia striata	Ligusticum filicinum
Agropyron repens	Ligusticum gravi
Alchemilla mollis	Ligusticum porteri
Alchemilla vulgaris	Lilium grayi
Aletris farinosa	Lilium philadelphicum
Alhagi camelorum	Linaria canadensis
Allium cernuum	Linaria dalmatica
Allium geyeri	Linaria vulgaris
Allium schoenoprasum	Linnaea borealis
Alnus incana	Linum lewisii
Aloe spp.	Linum medium
Aloe vera	Linum usitatissimum
Althea officinalis	Liquidambar orientalis
Amaranthus hybridus	Liquidamber styraciflua
Ambrosia ambrosioides	Lithospermum arvense

TABLE 2-continued

TABLE 2-continued		TABLE 2-continued		
Medicinal Plant	Medicinal Plant	Medicinal Plant	Medicinal Plant	
Ambrosia artemisiifolia	Lithospermum multiflorum	Asclepias subulata	Ocimum basilicum	
Ambrosia trifida	Lithospermum ruderale	Asclepias syriaca	Oenothera biennis	
Amelanchier alnifolia	Lobelia cardinalis	Asclepias texana	Oenothera hookeri	
Amsinckia intermedia	Lobelia cardinalis.	Asclepias tuberosa	Oplopanax horridum	
Amsonia hirtella	Lobelia cardinalis,	Asclepas viridis	Opuntia erinacea	
	Lobelia inflata			
Amygdalus persica		Asclepias viridis	Opuntia phaeacantha	
Anaphalis margaritacea	Lobelia kalmii	Asparagus officinale	Orobanche fasciculata	
Anemone deltoidea	Lobelia siphilitica	Aspidium filix-mas	Orobanche ludoviciana	
Anemone globosa	Lomatium cous	Astragalus gummifer	Orobanche uniflora	
Anemone halleri	Lomatium dissectum	Astragalus americanus	Osmorhiza obtusa	
Anemone occidentalis	Lophocereus (Pachycereus)	Astragalus membranaceus	Osmorrhiza longistylis	
Anemone patens	Lycium fremontii	Atriplex canescens	Osmorrhiza occidentalis	
Anemone patens,	Lycium pallidum	Avena fatua	Ourouparia gambir	
Anemone quinquefolia	Lycopodium clavatum	Avena sativa	Oxalis cymosa	
Anemone tuberosa	Lycopus americanus	Balsamorhiza sagittata	Oxalis oregana	
Anemopsis californica	Lycopus asper	Baptisia australis	Oxalis metcalfei	
Anethum graveolens	Lycopus uniflorus	Baptisia leucantha	Paeonia brownii	
Angelica sp.	Lycopus virginicus	Baptisia leucophaea	Paeonia californica	
Angelica archangelica	Lysichitum americanum	Baptisia sphaerocarpa	Panax quinquefolium	
Angelica arguta	Lythrum salicaria	Baptisia tinctoria	Panax trifolium	
Angelica dawsonii	Macromeria viridiflora	Buddleya sp.	Papaver rhoeas	
Angelica genuflexa	Magnolia grandiflora	Berberis fendleri	Papaver somniferum	
Angelica grayi	Mahonia aquifolia	Berberis vulgaris	Parthenium incanum	
Angelica hendersonii	Mahonia fremontii	Berberis -	Parthenocissus inserta	
Angelica lineariloba	Mahonia haematocarpa	Besseya wyomingensis	Parthenocissus quinquefolia	
Angelica pinnata	Mahonia nervosa	Bidens frondosa	Passiflora foetida	
Angelica venenosa	Mahonia repens	Bidens pilosa	Passiflora incarnata	
Antennaria howellii	Mahonia trifoliata	Bignonia capreolata	Passiflora lutea	
Antennaria rosea	Mahonia wilcoxii	Bouvardia ternifolia	Passiflora sanguinea	
Apocynum androsaemifolium	Malus sylvestris	Brassica arvensis	Paullinia cupana	
Apocynum cannabinum	Malva neglecta	Brickellia amplexicaulis	Pedicularis bracteosa	
Apocynum medium	Mammillaria arizonica	Brickellia californica	Pedicularis canadensis	
Aquilegia caerulea	Marah gilensis	Brickellia grandiflora	Pedicularis contorta	
Aquilegia chrysantha	Marrubium vulgare	Brugmansia sp.	Pedicularis densiflora	
Aralia californica	Matricaria chamomilla	Bryonia alba	Pedicularis gravii	
Aralia nudicaulis	Matricaria matricarioides	Bupleurum americanum		
			Pedicularis groenlandica	
Aralia racemosa	Medicago sativa	Bursera microphylla	Pedicularis lanceolata	
Aralia spinosa	Melampyrum lineare	Bursera odorata	Pedicularis parryi	
Arbutus menziesii	Melilotus albus	Cacalia decomposita	Pedicularis racemosa	
Arctium minus	Menispermum canadense	Caesalpinia gilliessii	Peganum harmala	
Arctostaphylos pungens	Mentha arvensis	Caesalpinia pulcherrima	Peniocereus greggii	
Arctostaphylos uva-ursi	Mentha pulegium	Caffea arabica	Penstemon cobaea	
		Calendula officinalis	Penstemon eatoni	
Argemone corymbosa	Mentha spicata			
Argemone mexicana	Menyanthes trifoliata	Callirhoe involucrata	Penstemon lyallii	
Argemone platyceras	Mertensia ciliata	Caltha biflora	Perezia nana	
Argemone polyanthemos	Mimulus guttatus	Caltha leptosepala	Perezia wrightii	
Arisaema atrorubens	Mirabilis longiflora	Caltha palustris	Perideridia gairdneri	
Arisaema dracontium	Mirabilis multiflorum	Calypso bulbosa	Perilla frutescens	
Arisaema stewardsonii	Mitchella repens	Camassia quamash	Petasites frigidus	
	Milchella repens Monarda citriodora			
Arisaema triphyllum		Camissonia (Oenothera)	Petasites frigidus,	
Aristolochia californica	Monarda didyma	Campsis radicans	Petasites sagittatus	
Aristolochia serpentaria	Monarda fistulosa	Cannabis sativa	Philadelphus lewisii	
Aristolochia watsonii	Monarda media	Capsella bursa-pastoris	Phoradendron flavescens	
Arnica angustifolium	Monarda menthaefolia	Capsicum annuum	Phoradendron juniperinum	
Arnica cordifolia	Monarda mollis	Capsicum frutescens	Physalis crassifolia	
Arnica latifolia	Monarda pectinata	Cardamine cordifolia	Physocarpus monogynus	
			Physostigma venenosum	
Arnica mollis	Monarda punctata	Carnegia gigantea		
Arnica montana	Monardella villosa	Cassia angustifolia	Phytolacca americana	
Artemisia douglasiana	Moneses uniflora	Cassia covesii	Picea engelmanni	
Artemisia filifolia	Monotropa hypopitys	Cassia fasciculata	Pinus contorta	
Artemisia franserioides	Monotropa uniflora	Cassia fistula	Pinus edulis	
Artemisia frigida,	Mortonia scabrella	Cassia leptocarpa	Pinus palustris	
Artemisia frigida	Myrica californica	Cassia narilandica	Pinus ponderosa	
Artemisia ludoviciana	Myrica cerifera	Cassia senna	Pinus strobus	
Artemisia tridentata	Myristica fragrans	Cassia wislizenii	Pinus taeda	
Artemisia vulgaris	Nelumbo lutea	Castanopsis chrysophylla	Piper sp	
Asarum canadense	Nepeta cataria	Castela emoryi	Piper cubeba	
Asarum caudatum	Nicotiana attenuata	Castilleja sp.	Plantago lanceolata	
Asclepias albicans	Nicotiana glauca	Castilleja miniata	Plantago major	
-				
Asclepias asperula	Nicotiana repanda	Caulophyllum thalictrioides	Plantago patagonica	
Asclepias brachystephana	Nicotiana tabacum	Ceanothus americanus	Plantago rugeli	
Asclepias erosa	Nicotiana trigonophylla	Ceanothus cuneatus	Pluchea camphorata	
Asclepias fascicularis	Nuphar luteum	Ceanothus fendleri	Podophyllum peltatum	

TABLE 2-continued

TABLE 2-continued

TABLE 2-continued		TABLE 2-continued		
Medicinal Plant	Medicinal Plant	Medicinal Plant	Medicinal Plant	
Ceanothus herbaceum	Polygala lutea	Crataegus succulenta	Sabatia campestris	
Ceanothus spinosus	Polygala obscura	Cucurbita foetidissima	Sabatia stellaris	
Ceanothus velutinus	Polygala paucifolia	Cupressus [°] arizonica	Sagittaria cuneata	
Celastrus scandens	Polygala senega	Cupressus macrocarpa	Sagittaria latifolia	
Celtis occidentalis	Polygonatum biflorum	Curcuma sp.	salix sp.	
Centaurium venustum	Polygonatum canaliculatum	Cuscuta gronovi	Salix discolor	
	Polygonum bistortioides	Cymopterus fendleri	Salvia apiana	
Cephaelis ipecacuanha				
Cephalanthus occidentalis	Polymnia spp	Cynanchum nigrum	Salvia azurea	
Cerastium arvense	Polymnia canadensis	Cynara sp.	Salvia clevelandii	
Cercis occidentalis	Polypodium glycyrriza	Cynoglossum officinale	Salvia columbariae	
Cercocarpus sp.	Polystichum munitum	Cypripedium sp.	Salvia greggii	
Cetraria islandica	Populus balsamifera	Cypripedium acaule	Salvia henryi	
Chamaelirium luteum	Populus fremontii	Cypripedium arietinum	Salvia lemmonii	
Chelidonium majus	Populus tremulioides	Cypripedium calceolus	Salvia leucophylla	
Chelone glabra	Portulaca oleracea	Cypripedium montanum	Salvia mellifera	
Chelone lyoni	Potentilla diversifolia	Cypripedium parviflorum	Salvia regla	
Chenopodium ambrosioides	Potentilla fruticosa	Cypripedium reginae	Salvia reflexa	
Chilopsis linearis	Potentilla palustris	Cytisus scoparius		
			Salvia spathaceae	
Chimaphila umbellata	Potentilla strigosa	Dalea formosa	Sambucus canadensis	
Chimaphila umbellata,	Potentilla tridentata	Darlingtonia californica	Sambucus mexicana	
Chionanthus virginiana	Proboscidea parviflora	Datura ferox	Sambucus racemosa	
Chlorogalum pomeridianum	Prosopis juliflora	Datura metelioides	Sanguinaria canadensis	
Chondrus crispus	Prunella vulgaris	Datura wrightii	Sanguisorba canadensis	
Choisya arizonica	Prunus americana	Daucus carota	Sanicula marilandica	
Chrysanthemum leucanthemum	Prunus avium	Delphinium barbeyi	Santalum album	
Chrysanthemum parthenium	Prunus laurocereus	Delphinium elongatum	Sanvitalia abertii	
Cichorium intybus	Prunus serotina	Dendromecon rigida	Sapindus saponaria	
Cicuta douglasii	Prunus virginiana	Dicentra canadensis	Saponaria officinalis	
Cimicifuga arizonica	Pseudotsuga menziesii	Dicentra cucullaria	Sarracenia psittacina	
Cimicifuga elata	Psoralea esculenta	Dicentra formosa	Sarracenia purpurea	
Cimicifuga racemosa	Ptelea pallida	Dicentra spectabilis	Sarracenia rubra	
Cinchona succirubra	Ptelea trifoliata	Digitalis purpurea	Sassafras IL	
Cinnamomum camphora	Pulsatilla ludoviciana	Dionaea muscipula	Satureja douglasii	
Cirsium undulatum	Punica granatum	Dioscorea villosa	Saururus cernuus	
Citrullus colocynthis	Purshia tridentata	Dipsacus sylvestris	Scopola carniolica	
Citrus sinensis	Pyrola asarifolia	Dipsacus fullonum	Scrophularia californica	
	Pyrola minor			
Claviceps purpurea		Dodecathion pulchellum	Scrophularia lanceolata	
Claytonia lanceolata	Pyrola rotundifolia	Dracocephalum moldavica	Scutellaria brittonii	
Clematis columbiana	Pyrola secunda	Dracocephalum parviflorum	Scutellaria californica	
Clematis hirsutissima	Prola virens	Drosera linearis	Scutellaria drummondii	
Clematis ligusticifolia	Quercus alba	Drosera rotundifolia	Scutellaria epilobiifolia	
Clematis pseudoalpina	Quercus gambelii	Dyssodia papposa	Scuteliaria galericulata	
Clematis viorna	Quillaja saponaria	Ecballium elaterium	Scutellaria incana	
Clematis virginiana	Ratibida columnaris	Echevaria rusbyi	Scutellaria integrifolia	
Cleome serrulata	Rhamnus alnifolia	Echinacea angustifolia	Scutellaria latiflora	
Cocculus sp.	Rhamnus betulifolia	Echinacea pallida	Scutellaria resinosa	
Cola nitida	Rhamnus californica	Echinacea purpurea	Scutellaria serrata	
Colchicum autumnale	Rhamnus frangula	Echinacea tennessiensis	Scutellaria tesselata	
Collinsonia canadensis	Rhamnus purshiana	Elettaria carmamomum	Scutellaria wrightii	
Commandra umbellata	Rheum officinale	Encelia farinosa	Sedum rhodanthum	
Conium maculatum	Rhus choriophylla	Ephedra californica	Sedum roseum	
Conopholis alpina	Rhus glabra	Ephedra nevadensis	Selenicereus spp.	
Conopholis americana	Rhus microphylla	Éphedra torreyana	Senecio aureus	
Convallaria majus	Rhus (Toxicodendron)	Ephedra trifurca	Senecio cineraria	
Convolvulus arvensis	Rhus trilobata	Ephedra viridis	Sequoia sempervirens	
Convolvulus scammonia	Ribes aureum	Epifagus virginianum	Serenoa repens	
Conyza canadense	Ricinus communis	Epigaea repens	Shephardia argentea	
Copaiba langsdorffii	Romneya coulteri	Epilobium angustifolium	Shephardia canadensis	
Coptis groenlandica	Rosa acicularis	Epilobium hirsutum	Sida hederacea	
Coptis laciniata	Rosa humilis	Epipactis gigantea	Sidalcea neomexicana	
Coptis occidentalis	Rosa virginiana	Epipactis helleborine	Sidalcea malvaeflora	
Corallorhiza maculata	Rosa woodsii	Equisetum arvense	Silphium laciniata	
Corallorrhiza striata	Rubus idaeus	Equisetum pratense	Silphium perfoliatum	
Cordia boissieri	Rubus odoratus	Eremocarpus setigerus	Silphium terebinthinaceum	
Cornus canadensis	Rubus parviflorus	Eriodictyon angustifolia	Silybum marianum	
			-	
Cornus florida	Rudbeckia hirta	Eriodictyon californica	Simmondsia chinensis	
Cornus stolonifera	Rudbeckia laciniata	Eriodictyon crassifolium	Smilacina racemosa	
Corydalis aureus	Ruellia ciliosa	Eriodictyon glutinosa	Smilacina stellata	
Corydalis sempervirens	Rumex acetosella	Eriogonum leptophyllum	Smilacina trifolia	
Crataegus spp.	Rumex crispus	Eriogonum umbellata	Smilax spp.	
Crataegus columbiana	Rumex hymenosepalus	Eriogonum wrightii	Smilax californica	
Crataegus douglasii	Ruta graveolens	Erodium cicutarium	Smilax glauca	
Crataegus mollis	Sabal texana	Eryngium leavenworthii	Smilax herbacea	

TABLE 2-continued

TABLE 2-continued

TABLE 2-continued		TABLE 2-continued		
Medicinal Plant	Medicinal Plant	Medicinal Plant	Medicinal Plant	
Eryngium yuccafolium	Solanum carolinense	Grindelia squarrosa	Vaccinium scoparium	
Erysimum capitatum	Solanum dulcamara	Guaiacum angustifolium	Vaccinium tenellum	
Erythronium grandiflorum	Solanum eleagnifolium	Guaiacum coulteri	Vaccinium uliginosum	
Erythronium montanum	Solanum nodiflorum	Guaiacum sanctum	Vaccinium vitis-idaea	
Erythroxylon coca	Solidago canadensis	Gutierrezia sarothrae	Valeriana acutiloba	
Eschscholtzia californica	Sophora secundiflora	Habenaria blephariglottis	Valeriana arizonica	
Eschscholtzia mexicana	Sorbus scopulina	Habeneria fimbriata	Valeriana edulus	
Eschscholtzia minutiflora	Spartium junceum	Habenaria (Plantanthera)	Valeriana officinalis	
Eucalyptus sp.	Sphaeralcea ambigua	Hagenia abyssinica	Valeriana occidentalis	
Euonymus occidentalis	Sphaeralcea angustifolia		Valeriana sitchensis	
Eupatorium coelestinum	Sphaeralcea coccinea	Hamamelis virginiana Haplopappus laricifolius		
Eupatorium greggii	Sphaeralcea fendleri		Vancouveria hexandra	
Eupatorium herbaceum	Sphaeralcea parviflora	Hedeoma hyssopifolium	Veratrum californicum	
Eupatorium maculatum	Sphenosciadium capitellatum	Hedeoma oblongifolia	Veratrum viride	
Eupatorium perfoliatum	Spigelia marilandica	Hedysarum alpinum	Verbascum blattaria	
Eupatorium purpureum	Spiraea alba	Helenium (Dugaldia)	Verbascum thapsus	
Eupatorium rugosum	Spiraea tomentosa	Heliotropium convolvulaceum	Verbena bipinnatifida	
Eustoma grandiflorum	Stachys albens	Heracleum lanatum	Verbena bracteata	
Eysenhardtia polystachya	Stachys palustris	Heterotheca grandiflora	Verbena canadensis	
Fallugia paradoxa	Stachys rigida	Heterotheca psammophylla	Verbena ciliata	
Ferula foetida	Stellaria media	Heterotheca subaxillaris	Verbena gooddingii	
Ferula galbaniflua	Stenocereus thurberi	Heuchera americanus	Verbena hastata	
Flourensia cernua	Sticta PH	Heuchera micrantha	Verbena macdougalii	
Fouquieria splendens	Stillingia sylvatica	Heuchera parvifolia	Verbena stricta	
Fragaria glauca	Streptopus amplexifolius	Heuchera sanguinea	Verbena wrightii	
Fragaria ovalis	Strychnos nux-vomica	Hibiscus moscheutos	0	
Fragaria virginiana	Swertia radiata		Verbesina encelioides	
Frankenia grandiflora	Symphytum officinalis	Hibiscus oculiroseus	Veronica americana	
Frankenia palmeri	Symplocarpus foetidus	Hierochloe odorata	Veronica chamaedrys	
Fraxinus ornus	Tanacetum huronense	Holodiscus dumosus	Veronicastrum IM	
Fremontia californica	Tanacetum parthenium	Humulus americanus	Viburnum acerifolium	
Fritillaria atropurpurea	Tanacetum vulgare	Humulus lupulus	Viburnum americanum	
Fritillaria pudica	Taraxacum sp.	Hydrastis canadensis	Viburnum cassinoides	
Fucus vesiculosus	Taxus brevifolia			
Fumaria officinalis	Tecoma stans	Hydrocotyle bonariensis	Viburnum edule	
Gaillardia pinnatifida	Teucrium laciniatum	Hydrophyllum capitatum	Viburnum ellipticum	
Galium aparine	Thalictrum fendleri	Hyocyamus niger	Viburnum opulus	
Galium borealis	Thamnosma texana	Hypericum ascyron	Viburnum prunifolium	
Garcinia hanburyi	Thamnosma montana	Hypericum aureum	Viburnum rufidulum	
Garrya spp.	Thelesperma gracile	Hypericum formosum	Vigueria dentata	
Garrya elliptica	Tephrosia virginiana	Hypericum perforatum	Vinca major	
Garrya flavescens	Thermopsis montana	Hyptis emoryi	Viola sp	
Garrya wrightii	Thuja plicata		-	
Gaultheria procumbens	Thymus vulgaris	Hyssopus officinalis	Viola canadensis	
Gaultheria shallon	Tillandsia recurvata	Ilex vomitoria	Viola pedata	
Gaura lindheimeri	Tillandsia usnioides	Impatiens biflora	Viola tricolor	
Gaura parviflora	Toluifera balsamum	Impatiens capensis	Vitex agnus-castus	
Gaylussacia brachycera	Toluifera pereirae	Impatiens pallida	Xanthium spinosum	
Gelsemium sempervirens	Toxicodendron radicans	Indigofera sphaerocarpa	Xanthium strumarium	
Gentiana affinis	Toxicodendron vernix	Inula helenium	Xerophyllum tenax	
Gentiana algida	Tradescantia occidentalis	Inuta netenium Ipomea arborescens	Yucca baccata	
Gentiana andrewsi	Tragopogon dubius	1		
Gentiana calycosa	Trautvettaria carolinensis	Ipomea jalapa	Yucca baileyi	
Gentiana crinata	Tribulus terrestrus	Ipomea leptophylla	Yucca elata	
Gentiana heterosepala	Trichostema lanatum	Iris missouriensis	Yucca schottii	
Gentiana parryi	Trifolium pratense	Iris prismatica	Zanthoxylum fagaria	
Gentiana saponaria	Trillium erectum	Iris versicolor	Zauschneria latifolia	
Gentiana simplex	Trillium grandiflorum	Jateorhiza palmata	Zigadenus elegans	
Gentiana thermalis	Trillium ovatum	Jatropha cardiophylla	0 0	
Gentianella (Gentian)	Trillium sessile		Zigadenus venenosus Zinnihan m	
Geranium maculatum	Trillium undulatum	Jatropha dioica	Zingiber sp.	
Geranium richardsonii	Trollius laxus	Jatropha macrorhiza	Zizia aptera	
Geranium viscosissimum	Tsuga mertensiana	Jeffersonia diphylla		
Geum rivale	Turnera diffusa			
Geum triflorum	Umbellularia californica			
Gigartina mamillosa	Urginea maritima			
Gillenia trifoliata	Urtica dioica	[0124] The dwarf phenoty	pe may be created using t	
Glecoma hederacea	Usnea barbata	cDNAs of the present inventi-		
	Usnea hirsutissima	*		
Glycyrriza glabra		variety of plant virus expres	sion vectors. The plant vir	
Glycyrriza glabra	Vaccinium corymbosum			
Glycyrriza glabra Glycyrrhiza lepidota	Vaccinium corymbosum Vaccinium mvrtillus	selected may depend on the	plant system chosen and	
Glycyrriza glabra Glycyrrhiza lepidota Gnaphallium sp.	Vaccinium myrtillus	selected may depend on the		
<i>Glycyrriza glabra Glycyrrhiza lepidota</i> Gnaphallium sp. Goodyera spp.	Vaccinium myrtillus Vaccinium ovatum	known susceptibility to viral	infection. Preferred embod	
Glycyrriza glabra Glycyrrhiza lepidota	Vaccinium myrtillus		infection. Preferred embod	

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TABLE 2-continued

TABLE 3		TABLE 3-continued		
Plant Viruses	Plant Viruses	Plant Viruses	Plant Viruses	
Abelia latent tymovirus	Lucerne transient streak	Carnation 1 alphacryptovirus	Petunia asteroid mosaic	
Abutilon mosaic bigeminivirus	Lychnis ringspot hordeivirus	Carnation 2 alphacryptovirus	Physalis mild chlorosis luteovirus	
	Maclura mosaic macluravirus	Carnation etched ring caulimovirus		
	Maize dwarf mosaic potyvirus	Carnation Italian ringspot	Pineapple chlorotic leaf streak	
	Maize streak monogeminivirus	Carnation latent carlavirus	Pineapple wilt-associated	
	Maracuja mosaic tobamovirus	Carnation mottle carmovirus Carnation mottle carmovirus	Pittosporum vein yellowing Plantain 6 carmovirus	
	Marigold mottle potyvirus Melandrium yellow fleck	Carnation necrotic fleck	Plantain 7 potyvirus	
	Melilotus mosaic potyvirus	Carnation ringspot dianthovirus	Plantain X potexvirus	
	Melon Ourmia ourmiavirus	Carnation vein mottle potyvirus	Plum American line pattern ilarviru	
	Melothria mottle potyvirus	Carnation yellow stripe necrovirus	Plum pox potyvirus	
Amaryllis alphacryptovirus	Milk vetch dwarf nanavirus	Carrot mosaic potyvirus	Poinsettia mosaic tymovirus	
	Mulberry latent carlavirus	Carrot mottle mimic umbravirus	Poplar mosaic carlavirus	
	Muskmelon vein necrosis carlavirus	Carrot mottle umbravirus	Poplar vein yellowing	
	Myrobalan latent ringspot nepovirus	Carrot yellow leaf closterovirus	Potato 14R tobamovirus	
	Nandina mosaic potexvirus	Cassava African mosaic	Potato A potyvirus	
	Narcissus late season yellows Narcissus latent macluravirus	Cassava brown streak potyvirus Cassava brown streak-associated	Potato Andean latent tymovirus Potato Andean mottle comovirus	
	Narcissus mosaic potexvirus	Cassava brown streak-associated Cassava Caribbean mosaic	Potato aucuba mosaic potexvirus	
	Narcissus tip necrosis carmovirus	Cassava Colombian symptomless	Potato black ringspot nepovirus	
	Narcissus tip necrosis carmovirus	Cassava common mosaic	Potato leafroll luteovirus	
	Narcissus yellow stripe potyvirus	Cassava green mottle nepovirus	Potato M carlavirus	
	Neckar River tombusvirus	Cassava Indian mosaic	Potato mop-top furovirus	
	Nerine potyvirus	Cassava Ivorian bacilliform	Potato mop-top furovirus	
	Nicotiana velutina mosaic furovirus	Cassava Ivorian bacilliform	Potato T trichovirus	
	Oat blue dwarf marafivirus	Cassava X potexvirus	Potato U nepovirus	
Asparagus 1 potyvirus	Oat blue dwarf marafivirus	Cassia mild mosaic carlavirus	Potato V potyvirus	
	Oat golden stripe furovirus	Cassia severe mosaic closterovirus	Potato X potexvirus	
	Odontoglossum ringspot	Celery latent potyvirus	Potato Y potyvirus	
	Okra leaf-curl bigeminivirus	celery mosaic potyvirus	Potato yellow dwarf	
	Okra mosaic tymovirus	Cherry leaf roll nepovirus	Primula mosaic potyvirus	
61	Olive latent 1 sobemovirus	Chickpea bushy dwarf potyvirus	Primula mottle potyvirus	
, I	Olive latent 2 ourmiavirus	Chickpea chlorotic dwarf	Prune dwarf ilarvirus	
	Onion mite-borne latent potexvirus	Chickpea distortion mosaic Chicory yellow mottle nepovirus	Prunus necrotic ringspot ilarvirus	
	Onion yellow dwarf potyvirus Orchid fleck rhabdovirus	Chilli veinal mottle potyvirus	Radish mosaic comovirus Raspberry ringspot nepovirus	
	Panicum mosaic sobemovirus	Chino del tomat, bigeminivirus	Red clover mottle comovirus	
	Papaya mosaic potexvirus	Citrus leaf rugose ilarvirus	Red clover necrotic mosaic	
	Papaya ringspot potyvirus	Citrus ringspot virus	Red clover vein mosaic carlavirus	
	Paprika mild mottle tobamovirus	Clover mild mosaic virus	Rhynchosia mosaic bigeminivirus	
Bean pod mottle comovirus	Parietaria mottle ilarvirus	Clover wound tumor phytoreovirus	Ribgrass mosaic tobamovirus	
Bean yellow mosaic potyvirus	Parsnip leafcurl virus	Clover wound tumor phytoreovirus	Rice hoja blanca tenuivirus	
	Parsnip mosaic potyvirus	Clover yellow mosaic potexvirus	Rice stripe necrosis furovirus	
	Parsnip yellow fleck sequivirus	Clover yellow vein potyvirus	Rice stripe tenuivirus	
	Passiflora ringspot potyvirus	Colocasia bobone disease	Rose tobamovirus	
	Passionfruit woodiness potyvirus	Commelina X potexvirus	Rubus Chinese seed-borne	
Beet necrotic yellow vein furovirus		Cowpea chlorotic mottle	saguaro cactus carmovirus	
	Pea early browning tobravirus Pea enation mosaic enamovirus	Cowpea mild mottle carlavirus	Scrophularia mottle tymovirus	
	Pea mild mosaic comovirus	Cowpea mosaic comovirus Cowpea mosaic comovirus	Shallot latent carlavirus Shallot mite-borne latent potexvirus	
	Pea mosaic potyvirus	Cowpea mosale comovirus	Shallot yellow stripe potyvirus	
	Pea seed-borne mosaic potyvirus	Cowpea severe mosaic comovirus	Silene X potexvirus	
	Pea streak carlavirus	Cowpea severe mosaic comovirus	Sint-Jan's onion latent carlavirus	
	Peach enation nepovirus	Croton yellow vein mosaic	Sitke waterborne tombusvirus	
	Peach rosette mosaic nepovirus	Cucumber green mottle mosaic	Solanum apical leaf curling	
	Peanut chlorotic streak caulimovirus	Cucumber leaf spot carmovirus	Solanum nodiflorum mottle	
Bramble yellow mosaic potyvirus	Peanut clump furovirus	Cucumber mosaic cucumovirus	Solanum nodiflorum mottle	
Broad bean mottle bromovirus	Peanut mottle potyvirus	Cucumber mosaic cucumovirus	Sonchus cytorhabdovirus	
	Peanut stunt cucumovirus	Cucumber necrosis tombusvirus	Sonchus yellow net	
	Peanut yellow spot tospovirus	Cycas necrotic stunt nepovirus	Sorghum mosaic potyvirus	
	Pelargonium flower break	Cymbidium ringspot tombusvirus	Sowbane mosaic sobemovirus	
	Pelargonium line pattern	Cynara nucleorhabdovirus	Soybean crinkle leaf bigeminivirus	
	Pelargonium vein clearing	Dahlia mosaic caulimovirus	Soybean dwarf luteovirus	
	Pelargonium zonate spot	Dandelion yellow mosaic	Soybean mild mosaic virus	
	Pepino mosaic potexvirus	sequivirus	Soybean mosaic potyvirus Spinach latent ilarvirus	
	Pepper Indian mottle potyvirus Pepper mild mosaic potyvirus	Daphne Y potyvirus Dasheen bacilliform badnavirus	Spinach latent liarvirus Spinach temperate alphacryptovirus	
	Pepper mild mottle tobamovirus	Dasheen mosaic potyvirus	Spring beauty latent bromovirus	
	Pepper Moroccan tombusvirus	Datura Colombian potyvirus	Statice Y potyvirus	
	Pepper motile potyvirus	Datura distortion mosaic potyvirus	Strawberry latent ringspot	
comercana mananna mosaic	Pepper ringspot tobravirus	Datura unstortion mosaic potyvnus Datura innoxia Hungarian mosaic	Subterranean clover red leaf	
Caper latent carlavirus		Datura mosaic notyvirus	Sugarcane mosaic notwirus	
Caper latent carlavirus Caraway latent nepovirus	Pepper severe mosaic potyvirus Pepper Texas bigeminivirus	Datura mosaic potyvirus Datura necrosis potyvirus	Sugarcane mosaic potyvirus Sunflower ringspot ilarvirus	

TABLE 3-continued

TABLE 3-continued

Plant Viruses	Pla
Datura yellow vein	Swe
Desmodium mosaic potyvirus	Swe
Dioscorea green banding mosaic	Swe
Dioscorea latent potexvirus	Swe
Dogwood mosaic nepovirus	Swe
Dulcamara mottle tymovirus Eggplant green mosaic potyvirus	Swe Swe
Eggplant mild mottle carlavirus	Tan
Eggplant mottled crinkle	Tan
Eggplant mottled dwarf	Telf
Eggplant severe mottle potyvirus	Tob
Elderberry carlavirus	Tob
Elderberry latent carmovirus Elm mottle ilarvirus	Tob Tob
Epirus cherry ourmiavirus	Tob
Erysimum latent tymovirus	Tob
Eucharis mottle nepovirus	Tob
Euphorbia mosaic bigeminivirus	Tob
Foxtail mosaic potexvirus	Tob
Foxtail mosaic potexvirus	Tob Tob
Foxtail mosaic potexvirus Frangipani mosaic tobamovirus	Tob
Furcraea necrotic streak	Tob
Galinsoga mosaic carmovirus	Tob
Garlic common latent carlavirus	Tob
Glycine mottle carmovirus	Tob
Grapevine A trichovirus	Tob
Grapevine ajinashika disease Grapevine Algerian latent	Tob Tob
Grapevine B trichovirus	Tob
Grapevine Bulgarian latent	Ton
Grapevine chrome mosaic	Ton
Grapevine chrome mosaic	Ton
Grapevine corky bark-associated	Ton
Grapevine fanleaf nepovirus Grapevine fleck virus	Ton Ton
Grapevine leafroll-associated	Ton
Grapevine line pattern ilarvirus	Ton
Grapevine stem pitting associated	Ton
Grapevine stunt virus	Ton
Groundnut chlorotic spot	Ton
Groundnut rosette umbravirus Guar top necrosis virus	Ton Ton
Habenaria mosaic potyvirus	Ton
Helenium S carlavirus	Tro
Henbane mosaic potyvirus	Tro
Heracleum latent trichovirus	Tul
Hibiscus latent ringspot nepovirus	Tul:
Hippeastrum mosaic potyvirus Honeysuckle latent carlavirus	Tul: Tul:
Hop American latent carlavirus	Tur
Hop latent carlavirus	Tur
Humulus japonicus ilarvirus	Tur
Hydrangea mosaic ilarvirus	Tur
Impatiens latent potexvirus	Ullu
Impatiens necrotic spot tospovirus Iris fulva mosaic potyvirus	Ullu Vall
Ivy vein clearing cytorhabdovirus	Van
Johnsongrass mosaic potyvirus	Vio
Kalanchoe isometric virus	Vio
Konjak mosaic potyvirus	Wat
Kyuri green mottle mosaic Lamium mild mottle fabavirus	Wat Wat
Lato River tombusvirus	Wee
Leek yellow stripe potyvirus	Wel
Lettuce big-vein varicosavirus	Wh
Lettuce infectious yellows	Wh
Lettuce mosaic potyvirus	Wh
Lettuce necrotic yellows	Wil
Lettuce speckles mottle umbravirus Lilac chlorotic leafspot capillovirus	Wil Wil
Lilac ring mottle ilarvirus	Win
Lily X potexvirus	Wis
Lisianthus necrosis necrovirus	Yar

nt Viruses veet clover latent veet clover necrotic mosaic veet potato feathery mottle eet potato latent potyvirus veet potato mild mottle veet potato ringspot nepovirus veet potato sunken vein marillo mosaic potyvirus mus latent potexvirus lfairia mosaic potyvirus pacco etch potyvirus bacco leaf curl bigeminivirus bacco mild green mosaic bacco mosaic satellivirus bacco mosaic tobamovirus bacco mottle umbravirus bacco necrosis necrovirus bacco necrosis satellivirus bacco necrotic dwarf luteovirus bacco rattle tobravirus bacco ringspot nepovirus pacco streak ilarvirus bacco stunt varicosavirus pacco vein mottling potyvirus bacco vein-distorting luteovirus bacco wilt potyvirus oacco yellow dwarf bacco yellow net luteovirus bacco yellow vein umbravirus bacco yellow vein assistor mato aspermy cucumovirus mato Australian leafcurl mato black ring nepovirus mato black ring nepovirus mato bushy stunt tombusvirus mato golden mosaic mato mild mottle potyvirus mato mosaic tobamovirus mato mottle bigeminivirus mato Peru potyvirus mato ringspot nepovirus mato spotted wilt tospovirus mato top necrosis nepovirus mato yellow leaf curl opaeolum 1 potyvirus paeolum 2 potyvirus lare apple mosaic ilarvirus lip chlorotic blotch potyvirus lip halo necrosis virus ip X potexvirus rnip crinkle carmovirus rnip mosaic potyvirus rnip rosette sobemovirus rnip yellow mosaic tymovirus lucus mild mottle tobamovirus lucus mosaic potyvirus llota mosaic potyvirus nilla necrosis potyvirus ola mottle potexvirus ola mottle potexvirus atercress yellow spot virus atermelon mosaic 1 potyvirus atermelon mosaic 2 potyvirus eddel waterborne carmovirus elsh onion yellow stripe heat soil-borne mosaic furovirus heat streak mosaic rymovirus hite clover mosaic potexvirus ild cucumber mosaic tymovirus ild potato mosaic potyvirus ild potato mosaic potyvirus ineberry latent virus steria vein mosaic potyvirus m mosaic potyvirus

TABLE 3-continued

Plant Viruses	Plant Viruses
Lucerne Australian latent nepovirus	Zygocactus Montana X potexvirus
Lucerne Australian symptomless Lucerne enation nucleorhabdovirus	

[0125] A further listing of plants and plant viruses that may used with the methods of the invention is shown in Table 4. Additional examples of virus infections of plant species can be found at: http://image.fs.uidaho.edu/vide/. Additional virus accessions can be retrieved at: http://www.atcc.org.

TABLE 4

Plant or Virus Name	Plant or Virus Name
Cryptomeria japonica	Tulip band-breaking
Eucalyptus grandis	potyvirus
Eucalyptus nitens	Tulip breaking potyvirus
Eucalyptus urophylla	Tulip chlorotic blotch
Picea abies	potyvirus
Picea glauca	Tulip halo necrosis (?) virus
Pinus albicaulis	Tulip X potexvirus
Pinus aristata	Linum usitatissimum
Pinus armandii	Synonyms:
Pinus attenuata	Linum crepitans; Linum
Pinus ayacahuite	humile; Linum usitatissimum ssp.
Pinus balfouriana	transitorium; Linum usitatissimum
Pinus brutia	var. <i>humile</i>
Pinus bungeana	Common names:
Pinus canariensis	Flax; Linseed; Lino
Pinus cembroides	Susceptible to:
Pinus contorta	Alfalfa mosaic alfamovirus
Pinus culminicola	Beet curly top
Pinus durangensis	hybrigeminivirus
Pinus echinata	Beet pseudo-yellows (?)
Pinus edulis	closterovirus
Pinus elliottii	Oat blue dwarf marafivirus
Pinus engelmannii	Tobacco rattle tobravirus
Pinus flexilis	Hibiscus
Pinus gerardiana	Susceptible to:
Pinus griffithii	Abutilon mosaic
Pinus halepensis	bigeminivirus
Pinus hartwegii	Cotton leaf crumple
Pinus jefferyi	bigeminivirus
Pinus koraiensis	Hibiscus yellow mosaic (?)
Pinus lambertiana	tobamovirus
Pinus lumholtzii	Hibiscus cannabinus
Pinus massoniana	Common names:
Pinus monticola	Deccan-hemp; Indian-hemp;
Pinus mugo	Kenaf
Pinus palustris	Susceptible to:
Pinus pinaster	Cotton anthocyanosis (?)
Pinus pinceana	luteovirus
Pinus ponderosa	Cotton leaf crumple
Pinus pungens	bigeminivirus
Pinus radiata	Cotton leaf curl
Pinus resinosa	bigeminivirus
Pinus roxburghii	Hibiscus chlorotic ringspot
Pinus sabiniana	carmovirus
Pinus serotina	Hibiscus latent ringspot
Pinus strobus	nepovirus
Pinus sylvestris	Kenaf vein-clearing (?)
Pinus tabulaeformis Pinus taada	rhabdovirus Melye yein electring
Pinus taeda Binus thumborgii	Malva vein clearing
Pinus thunbergii Binus torrenang	potyvirus Olaro mosoia tumovirus
Pinus torreyana	Okra mosaic tymovirus
Pinus virginiana	Ficus carica
Pinus wangii Binus wanging	Common names:
Pinus yunnanensis	Fig; Higo

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	
Populus deltoides	Susceptible to:	Susceptible to:	potexvirus	
Populus tremuloides	Fig (?) potyvirus	Poplar mosaic carlavirus	Cymbidium ringspot	
Cryptomeria japonica	Fig S carlavirus	Poplar vein yellowing (?)	tombusvirus	
Eucalyptus grandis Eucalyptus nitens	Morus alba Synonyms:	nucleorhabdovirus Populus candicans	Cymbidium alexanderi	
Eucalyptus nitens Eucalyptus urophylla	Morus alba f. tatarica;	Synonyms:	Susceptible to: Odontoglossum ringspot	
Picea abies	Morus alba var.	Populus balsamifera ssp.	tobamovirus	
Picea glauca	constantinopolitana; Morus alba	balsamifera; Populus tacamahacca		
Pinus albicaulis	var. multicaulis; Morus indica;	Common names:	Synonyms:	
Pinus aristata	Morus multicaulis	Balsam poplar; Tacamahac	Rossioglossum grande	
Pinus armandii	Common names:	poplar; Balm of Gilead	Susceptible to:	
Pinus attenuata Pinus avacaluite	White mulberry; Mora Susceptible to:	Susceptible to: Poplar mosaic carlavirus	Odontoglossum ringspot tobamovirus	
Pinus ayacahuite Pinus balfouriana	Citrus enation- woody gall	Populus deltoides subspecies	Cocos nucifera	
Pinus brutia	(?) luteovirus	angulata, monilifera,	Common names:	
Pinus bungeana	Mulberry latent carlavirus	missouriensis	Coconut; Coconut palm;	
Pinus canariensis	Mulberry ringspot	Susceptible to:	Copra; Khopra; Nariyal; Coco	
Pinus cembroides	nepovirus	Poplar mosaic carlavirus	Susceptible to:	
Pinus contorta	Mirabilis jalapa	Ulmus americana	Coconut foliar decay	
Pinus culminicola	Common names:	Common names:	nanavirus	
Pinus durangensis	Common four-o'clock	American elm	Papaver nudicaule	
Pinus echinata Pinus edulis	Susceptible to: Mirabilis mosaic	Susceptible to: Cherry leaf roll nepovirus	Synonyms: Papaver miyabeanum	
Pinus elliottii	caulimovirus	Ulmus glabra	Common names:	
Pinus engelmannii	Fraxinus excelsior	Synonyms:	Iceland poppy; Arctic poppy	
Pinus flexilis	Synonyms:	Úlmus montana; Ulmus	Susceptible to:	
Pinus gerardiana	Fraxinus excelsior var.	scabra	Beet curly top	
Pinus griffithii	pendula	Common names:	hybrigeminivirus	
Pinus halepensis	Common names:	Scotch elm; Wych elm	Tobacco mosaic	
Pinus hartwegii	European ash	Susceptible to:	tobamovirus	
Pinus jefferyi Pinus koraiensis	Susceptible to:	Elm mottle ilarvirus	Tomato spotted wilt	
Pinus lambertiana	Arabis mosaic nepovirus Jasminum officinale	Ulmus minor Synonyms:	tospovirus Turnip mosaic potyvirus	
Pinus lumholtzii	Common names:	Ulmus campestris; Ulmus	Papaver somniferum	
Pinus massoniana	Poet's jasmine; Common	carpinifolia; Ulmus carpinifolia	Common names:	
Pinus monticola	jasmine; Jessamine	var. suberosa; Ulmus foliacea	Opium poppy	
Pinus mugo	Susceptible to:	Ulmus foliacea var. suberosa;	Susceptible to:	
Pinus palustris	Arabis mosaic nepovirus	Ulmus glabra var.	Bean yellow mosaic	
Pinus pinaster	Ligustrum vulgare	suberosa; Ulmus nitens;	potyvirus	
Pinus pinceana	Synonyms:	Ulmus suberosa	Papaver rhoeas	
Pinus ponderosa Pinus punceus	Ligustrum insulare; Ligustrum insulense	Susceptible to: Elm mottle ilarvirus	Common names:	
Pinus pungens Pinus radiata	Common names:	Subject: turf	Corn poppy; Shirley poppy; Field poppy	
Pinus resinosa	Common privet	Agropyron cristatum	Susceptible to:	
Pinus roxburghii	Susceptible to:	Festuca arizonica	Beet western yellows	
Pinus sabiniana	Arabis mosaic nepovirus	Agropyron cristatum x	clostreovirus	
Pinus serotina	Petunia asteroid mosaic	desertorum	Sesamum indicum	
Pinus strobus	tombusvirus	Festuca arundinacea	Synonyms:	
Pinus sylvestris	Olea europaea	Agropyron dasystachyum	Sesamum orientale	
Pinus tabulaeformis Pinus taeda	Common names: Olive; Aceituna	Festuca duriuscula Agropyron desertorum	Common names: Sesame; Benne seed	
Pinus thunbergii	Susceptible to:	Festuca eliator	Susceptible to:	
Pinus torreyana	Cherry leaf roll nepovirus	Agropyron elongatum	Abelia latent tymovirus	
Pinus virginiana	Olive latent ringspot	Festuca eliator	Apple stem pitting virus	
Pinus wangii	nepovirus	arundinacea	Arracacha A nepovirus	
Pinus yunnanensis	Olive latent 1 (?)	Agropyron inerme	Asparagus 3 potexvirus	
Populus deltoides	sobemovirus	Festuca idahoensis	Asystasia gangetica mottle (?)	
Populus tremuloides	Olive latent 2 (?)	Agropyron intermedium	potyvirus	
Populus trichocarpa	ourmavirus	Festuca longifolia	Blackgram mottle (?)	
Pseudotsuga menziesii Faxus brevifolia	Oenothera biennis Synonyms:	Agropyron riparium Festuca megalura	carmovirus Cassia yellow spot	
Ilmus parvifolia	<i>Oenothera biennis</i> ssp.	Agropyron sibericum	potyvirus	
Chamaecyparis lawsoniana	sulfurea; Oenothera chicagoensis;	Festuca ovina	Cherry leaf roll nepovirus	
Common names:	Oenothera muricata; Oenothera	Agropyron smithii	Citrus ringspot virus	
Port Orford-cedar; Ginger-	suaveolens; Onagra biennis	Festuca rubra	Lisianthus necrosis (?)	
oine; Oregon-cedar; Lawson's	Common names:	Agropyron spicatum	necrovirus	
cypress	Common evening-primrose;	Festuca rubra var.	Malva veinal necrosis (?)	
Susceptible to:	German rampion	commutata	potexvirus	
Arabis mosaic nepovirus	Insusceptible to:	Agropyron spicatum x	Melothria mottle (?)	
E <i>ucalyptus cloeziana</i> Common names:	Carnation vein mottle potyvirus	repens Festuca rubra var. rubra	potyvirus Mulberry latent carlavirus	
Cloeziana gum; Gympie	Cymbidium	Agropyron trachycaulum	Mulberry ringspot	
			ALANCONI & ILLEDPOL	
messmate	Susceptible to:	Hordeum brachyantherum	nepovirus	

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TABLE 4-continued

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	
Koeleria cristata	Patchouli mottle (?)	Buchloe dactyloides	tombusvirus	
Agrostis alba	potyvirus	Sporobolus airoides	Dogwood mosaic (?)	
Lolium multiflorum	Pea stem necrosis virus	Calamovilfa longifolia	nepovirus	
Agrostis palustris Lolium perenne	Peach enation (?) nepovirus Peanut green mosaic	Sporobolus crypatandrus Cynodon dactylon	Elm mottle ilarvirus Melon Ourmia ourmiavirus	
Agrostis tenuis	potyvirus	LEGUMES	Okra mosaic tymovirus	
Oryzopsis hymenoides	Peanut mottle potyvirus	Astragalus cicer	Poplar mosaic carlavirus	
Alopecurus arundinaceus	Peanut stunt cucumovirus	Onobrychis viciaefolia	Prune dwarf ilarvirus	
Phalaris arundinacea	Satsuma dwarf (?)	Coronilla varia	Ribgrass mosaic	
Alopecurus pratensis	nepovirus	Trifolium hybridum	tobamovirus	
Phleum alpinum	Soybean mild mosaic virus	Hedysarum boreale	Spinach latent ilarvirus	
Arcatagrostis latifolia Phlaum pratansa	Sweet potato yellow dwarf (?) ipomovirus	Trifolium pratense Lotus corniculatus	Strawberry latent ringspot (?) nepovirus	
Phleum pratense Beckmannia syzigachne	Tobacco ringspot nepovirus	Trifolium repens	Sweet potato mild mottle	
Phragmites australis	Watermelon mosaic 2	Lupinus spp.	ipomovirus	
Bromus biebersteinii	potyvirus	Trifolium repens L.	Tobacco ringspot nepovirus	
Poa alpina	Phytolacca americana	Medicago sativa	Tobacco streak ilarvirus	
Bromus carinatus	Synonyms:	Vicia villosa	Tomato spotted wilt	
Poa ampla	Phytolacca decandra	Melilotus officinalis	tospovirus	
Bromus catharticus	Common names:	Tritolium ambigium	Polypodium vulgare	
Poa bulbosa	Pokeweed; Poke;	Astragalus glycyphyllos	Susceptible to:	
Bromus inermis	Pigeonberry	Common names:	Fern (?) potyvirus	
Poa canbyi Bromus marginatus	Susceptible to: Alfalfa mosaic alfamovirus	Liquorice milk-vetch	rimula malacoides	
Bromus marginatus	Bean yellow mosaic	Susceptible to: Alfalfa mosaic alfamovirus	Susceptible to: Carnation mottle	
Poa compressa Bromus mollis	potyvirus	Alfalia mosaic alfamovirus Astragalus sinicus	carmovirus	
Poa glauca	Beet curly top	Susceptible to:	Hydrangea ringspot	
Dactylis glomerata	hybrigeminivirus	Bean leaf roll luteovirus	potexvirus	
Poa palustris	Beet mosaic potyvirus	Milk vetch dwarf nanavirus	Primula mottle (?) potyvirus	
Deschampsia caespitosa	Carnation mottle	Soybean dwarf luteovirus	Sweet potato mild mottle	
Poa pratensis	carmovirus	Subterranean clover red leaf	ipomovirus	
Viruses for Graminae:	Carnation ringspot	luteovirus	Viola mottle potexvirus	
Maize streak monogeminivirus	dianthovirus	Subterranean clover stunt	Pteris 'Childsii'	
Wheat streak mosaic rymovirus	Cucumber mosaic	nanavirus Wata mala a maria 2	Susceptible to:	
Barley yellow dwarf luteovirus Barley stripe mosaic hordeivirus	cucumovirus Cymbidium ringspot	Watermelon mosaic 2 potyvirus	Harts tongue fern (?) tobravirus	
Sugarcane mosaic potyvirus	tombusvirus	Coronilla varia	Ranunculus repens	
Beet western yellows luteovirus	Pepper veinal mottle	Synonyms:	Common names:	
Maize dwarf mosaic potyvirus	potyvirus	Securigera varia	Creeping buttercup	
Foxtail mosaic potexvirus	Pokeweed mosaic potyvirus	Common names:	Susceptible to:	
Johnsongrass mosaic potyvirus	Red clover necrotic mosaic	Crown-vetch; Trailing	Arabis mosaic nepovirus	
Panicum mosaic (?) sobemovirus	dianthovirus	crown-vetch	Ranunculus repens	
Rice stripe tenuivirus	Tobacco rattle tobravirus	Susceptible to:	symptomless (?) rhabdovirus	
Rice hoja blanca tenuivirus	Tobacco ringspot nepovirus	Peanut stunt cucumovirus	Malus domestica	
Wheat yellow leaf closterovirus Brome mosaic bromovirus	Tomato black ring nepovirus	Trifolium hybridum Common names:	Synonyms: Malus malus Purus malus	
Ribgrass mosaic tobamovirus	Turnip mosaic potyvirus	Alsike clover; Swedish	Malus malus; Pyrus malus Common names:	
Wheat soil-borne mosaic furovirus		clover; Trefle-hybride; Trefle-	Apple; Common apple	
Deschampsia caespitosa (L.)	Common names:	batard; Schwedenklee;	Susceptible to:	
Beauv. ssp. Beringensis	Common plantain;	Bastardklee; Trevo-hibrido;	Apple mosaic ilarvirus	
Poa sandbergii	Broadleaf plantain; Great plantain	Trebol-hibrido	Insusceptible to:	
Elymus angustus	Susceptible to:	Susceptible to:	Plum pox potyvirus	
Poa trivialis	Carnation vein mottle	Alfalfa mosaic alfamovirus	Malus platycarpa	
Elymus canadensis	potyvirus	Alsike clover vein mosaic	Susceptible to:	
Puccinellia distans	Cherry rasp leaf nepovirus	virus Roor loof roll lutoovirus	Apple chlorotic leaf spot trichovirus	
Elymus cinereus Secale cereale	Plantago 4 (?) caulimovirus Plantago mottle tymovirus	Bean leaf roll luteovirus Bean yellow mosaic	Apple stem pitting virus	
Elymus dahuricus	Ribgrass mosaic	potyvirus	Malus sylvestris	
Sitanion hystrix	tobamovirus	Beet curly top	Common names:	
Elymus glaucus	Phlox drummondii	hybrigeminivirus	Crab apple; Wild apple	
Stipa comata	Common names:	Beet yellows closterovirus	Susceptible to:	
Elymus junceus	Drummond phlox; Annual	Broad bean mottle	Apple chlorotic leaf spot	
Stipa viridula	phlox	bromovirus	trichovirus	
Elymus triticoides	Susceptible to:	Broad bean stain comovirus	Apple stem grooving	
Triticum aestivum, spp.	Apple mosaic ilarvirus	Clover mild mosaic virus	capillovirus	
WARM SEASON GRASSES	Arabis mosaic nepovirus	Clover yellow mosaic	Apple stem pitting virus	
Andropogon geradii Distichlis stricta	Beet curly top	potexvirus Clover vellow vein	Cherry rasp leaf nepovirus Horseradish latent	
Distichlis stricta Andropogon hallii	hybrigeminivirus Beet western yellows	Clover yellow vein potyvirus	caulimovirus	
Panicum virgatum	luteovirus	Cucumber mosaic	Tomato ringspot nepovirus	
Bouteloua curtipendula	Carnation ringspot	cucumovirus	Tulare apple mosaic	
			ilarvirus	
Schizachyrium scoparium	dianthovirus	Muskmelon vein necrosis	Harvirus	
Schizachyrium scoparium Bouteloua gracillis	Cherry leaf roll nepovirus	carlavirus	Prunus avium	

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TABLE 4-continued

tobamovirus

luteovirus

potyvirus

potyvirus

Synonyms:

Susceptible to:

potyvirus

bromovirus

carmovirus

carlavirus

potyvirus

potexvirus

potyvirus

nepovirus

cucumovirus

sobemovirus

enamovirus

potyvirus

comovirus

dianthovirus

carlavirus

nanavirus

Beet curly top

TABLE 4-continued

Plant or Virus Name tobravirus Pea enation mosaic enamovirus Pea streak carlavirus Peanut stunt cucumovirus Red clover mottle comovirus Red clover vein mosaic carlavirus Soybean dwarf luteovirus Subterranean clover red leaf luteovirus Tomato ringspot nepovirus Turnip mosaic potyvirus White clover mosaic potexvirus Lotus corniculatus Synonyms: Lotus corniculatus ssp. major; Lotus corniculatus var. major; Lotus major Common names Bird's-foot trefoil Susceptible to: Cucumber mosaic cucumovirus Lupinus albus Common names: White lupine; Egyptian lupine Susceptible to: Alfalfa mosaic alfamovirus Amaranthus leaf mottle potyvirus Bean common mosaic potyvirus Bean yellow mosaic potyvirus Beet western yellows luteovirus Bidens mosaic potyvirus Broad bean mottle bromovirus Broad bean true mosaic comovirus Carnation yellow stripe (?) necrovirus Cassia mild mosaic (?) carlavirus Chicory yellow mottle nepovirus Cowpea chlorotic mottle bromovirus Cucumber mosaic cucumovirus Dogwood mosaic (?) nepovirus Epirus cherry ourmiavirus Glycine mottle (?) carmovirus Lucerne Australian latent nepovirus Lucerne transient streak sobemovirus Pea enation mosaic enamovirus Pea streak carlavirus Peanut mottle potyvirus Peanut stunt cucumovirus Pepper Moroccan tombusvirus Plum pox potyvirus Prunus necrotic ringspot ilarvirus

Plant or Virus Name Cerasus avium var. aspleniifolia: Prunus avium var. aspleniifolia; Prunus cerasus var. avium Common names: Mazzard cherry; Sweet cherry Susceptible to: Arabis mosaic nepovirus Cherry leaf roll nepovirus Cherry mottle leaf (?) trichovirus Cherry rasp leaf nepovirus Epirus cherry ourmiavirus Myrobalan latent ringspot nepovirus Petunia asteroid mosaic tombusvirus Prunus domestica Common names: Plum Susceptible to: Apple chlorotic leaf spot trichovirus Arabis mosaic nepovirus Citrus enation-woody gall (?) luteovirus Petunia asteroid mosaic tombusvirus Plum American line pattern ilarvirus Plum pox potyvirus Prune dwarf ilarvirus Sowbane mosaic sobemovirus Strawberry latent ringspot (?) nepovirus Prunus persica Synonyms: Amygdalus persica; Amygdalus persica var. camelliiflora; Amygdalus persica var. densa; Persica vulgaris; Prunus persica var. camelliiflora; Prunus persica var. densa Common names: Peach; Melocotonero; Abridor; Durazno Susceptible to: Apple chlorotic leaf spot trichovirus Arabis mosaic nepovirus Cherry leaf roll nepovirus Cherry mottle leaf (?) trichovirus Cherry rasp leaf nepovirus Myrobalan latent ringspot nepovirus Peach enation (?) nepovirus Peach rosette mosaic nepovirus Peach yellow leaf (?) closterovirus Plum American line pattern ilarvirus Plum pox potyvirus Prune dwarf ilarvirus Prunus necrotic ringspot ilarvirus Strawberry latent ringspot (?) nepovirus Tomato ringspot nepovirus Pvrus communis Synonyms:

TABLE 4-continued

Plant or Virus Name Plant or Virus Name Ribgrass mosaic Pyrus asiae-mediae; Pyrus balansae; Pyrus bourgaeana; Sovbean dwarf luteovirus Pvrus domestica: Pvrus elata: Pyrus medvedevii Soybean mild mosaic virus Soybean mosaic potyvirus Common names: Subterranean clover red leaf Pear; Pera Susceptible to: Turnip mosaic potyvirus Apple chlorotic leaf spot Watermelon mosaic 2 trichovirus Apple stem pitting virus Wisteria vein mosaic Rosa Susceptible to: Apple mosaic ilarvirus Medicago sativa Arabis mosaic nepovirus Medicago caerulea var. pauciflora; Medicago Citrus enation - woody gall ?) luteovirus karatschaica; Medicago lavrenkoi; Prunus necrotic ringspot Medicago pauciflora; Medicago ilarvirus sativa var. pilifera Rose (?) tobamovirus Strawberry latent ringspot Alfalfa 1 alphacryptovirus (?) nepovirus Alfalfa 2 (?) betacryptovirus Rubus fruticosus Alfalfa mosaic alfamovirus Synonyms: Bean leaf roll luteovirus Rubus plicatus; Rubus Bean yellow mosaic affinis Common names: Blackberry; Bramble; European blackberry hybrigeminivirus Susceptible to: Broad bean mottle Black raspberry necrosis Carnation mottle virus Raspberry leaf curl (?) Carrot mosaic (?) potyvirus luteovirus Cassia mild mosaic (?) Strawberry latent ringspot (?) nepovirus Chickpea distortion mosaic Rubus idaeus Synonyms: Clover yellow mosaic Rubus buschii; Rubus idaeus var. vulgatus; Rubus Clover yellow vein vulgatus var. buschii Common names: Cucumber mosaic European red raspberry; Red raspberry Susceptible to Lucerne Australian latent Arabis mosaic nepovirus Lucerne Australian Black raspberry necrosis symptomless (?) nepovirus virus Lucerne enation (?) Cherry leaf roll nepovirus nucleorhabdovirus Cole latent (?) carlavirus Lucerne transient streak Raspberry bushy dwarf idaeovirus Milk vetch dwarf nanavirus Raspberry leaf curl (?) Narcissus mosaic potexvirus luteovirus Pea enation mosaic Raspberry ringspot nepovirus Raspberry vein chlorosis (?) nucleorhabdovirus Rubus yellow net (?) Pea seed-borne mosaic Pea streak carlavirus badnavirus Peanut stunt cucumovirus Red clover mottle Strawberry latent ringspot (?) nepovirus Thimbleberry ringspot virus Red clover necrotic mosaic Tomato ringspot nepovirus Red clover vein mosaic Citrus limon Synonyms: Subterranean clover stunt Citrus limonum; Citrus medica var. limon Tobacco ringspot nepovirus Common names: Tobacco streak ilarvirus Lemon; Limonero; Tobacco yellow dwarf Limoniere; Citronnier; monogeminivirus Zitronenbaum Watermelon mosaic 2 Susceptible to: Citrus enation - woody gall potyvirus White clover mosaic (?) luteovirus

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	
ootexvirus	Citrus leaf rugose ilarvirus	Shamrock	Papaya mosaic potexvirus	
Melilotus albus	Citrus ringspot virus	Susceptible to:	Pea streak carlavirus	
Synonyms:	Citrus tatter leaf capillovirus	Alfalfa mosaic alfamovirus	Peanut clump furovirus	
Melilotus albus var. annuus;	Citrus tristeza closterovirus	Bean leaf roll luteovirus	Pepper Moroccan	
Melilotus leucanthus	Citrus variegation ilarvirus	Peanut stunt cucumovirus	tombusvirus	
Common names:	Citrus paradisi	Soybean dwarf luteovirus	Plantago mottle tymovirus	
White sweet-clover; White	Common names:	Subterranean clover stunt	Poplar mosaic carlavirus	
melilot; Hubam	Grapefruit; Pomelo; Toronja	nanavirus	Prune dwarf ilarvirus	
Susceptible to:	Susceptible to:	WETLAND - RIPARIAN	Prunus necrotic ringspot	
Alfalfa mosaic alfamovirus	Citrus enation - woody gall	Agrostis alba	ilarvirus	
Apple mosaic ilarvirus	(?) luteovirus	Glyceria occidentalis	Red clover necrotic mosaic	
Bean common mosaic	Citrus leaf rugose ilarvirus	Alopecurus arundinaceus	dianthovirus	
ootyvirus	Citrus ringspot virus	Glyceria striata	Red clover vein mosaic	
Bean yellow mosaic	Citrus tristeza closterovirus	Alopecurus pratensis	carlavirus	
ootyvirus	Pepper veinal mottle	Hordeum brachyantherum	Rubus Chinese seed-borne	
Beet curly top	potyvirus	Beckmannia syzigachne	(?) nepovirus	
nybrigeminivirus	Citrus sinensis	Phalaris arundinacea	Scrophularia mottle	
Broad bean mottle	Synonyms:	Deschampsia caespitosa	tymovirus	
promovirus	Citrus aurantium var.	Poa palustris	Soybean mild mosaic virus	
Broad bean necrosis	sinensis; Citrus macracantha	WILDFLOWERS AND	Soybean mosaic potyvirus	
urovirus	Common names:	FORBES	Spinach latent ilarvirus	
Broad bean stain comovirus	Sweet orange; Naranja	Achillea millefolium	Strawberry latent ringspot	
Broad bean true mosaic	Susceptible to:	Lupinus albicalus	(?) nepovirus	
comovirus	Citrus enation - woody gall	Cheiranthus allionii	Tamus latent (?) potexvirus	
Clover yellow mosaic	(?) luteovirus	Lupinus perennis	Tobacco necrosis necrovirus	
ootexvirus	Citrus leaf rugose ilarvirus	Coreopsis lanceolata	Tobacco rattle tobravirus	
Clover yellow vein	Citrus leprosis (?)	Papaver rhoeas	Tobacco ringspot nepovirus	
ootyvirus	rhabdovirus	Echinacea purpurea	Tobacco streak ilarvirus	
Cucumber mosaic	Citrus ringspot virus	Ratibida columnaris	Tomato black ring	
cucumovirus	Citrus tatter leaf capillovirus	Eschscholtzia californica	nepovirus	
Galinsoga mosaic	Citrus tristeza closterovirus	Rudbeckia hirta	Tomato bushy stunt	
carmovirus	Sambucus canadensis	Linum lewisii	tombusvirus	
Milk vetch dwarf nanavirus	Common names:	Lupinus luteus	Viola mottle potexvirus	
Muskmelon vein necrosis	American elder; American	Common names:	White clover mosaic	
carlavirus	elderberry; Sweet elder	European yellow lupine;	potexvirus	
Pea enation mosaic	Susceptible to:	Yellow lupine	Scrophularia nodosa	
enamovirus	Elderberry carlavirus	Susceptible to:	Common names:	
Pea mild mosaic comovirus	Elderberry latent (?)	Bean yellow mosaic	Figwort; Figwort herb	
Pea streak carlavirus	carmovirus	potyvirus	Susceptible to:	
Peanut clump furovirus	Dodonaea viscosa	Clover yellow vein	Scrophularia mottle	
Peanut stunt cucumovirus	Common names:	potyvirus	tymovirus	
Plum pox potyvirus	Hop shrub	Dogwood mosaic (?)	Capsicum annuum	
Prune dwarf ilarvirus	Susceptible to:	nepovirus	Synonyms:	
Prunus necrotic ringspot	Dodonaea yellows-	Peanut stunt cucumovirus	Capsicum cordiforme	
larvirus	associated virus	Cheiranthus cheiri	Common names:	
Red clover mottle	Antirrhinum majus		Pimiento; Bell pepper;	
comovirus	Common names:	Synonyms: Erysimum cheiri		
Red clover vein mosaic	Snapdragon	2	Cayenne pepper; Chili pepper;	
	1 0	Common names:	Common garden pepper; Green	
arlavirus Subtorronoon olovor stunt	Susceptible to:	Wallflower	pepper; Mango pepper; Paprika	
Subterranean clover stunt	Alfalfa mosaic alfamovirus	Susceptible to:	pepper Susceptible to:	
nanavirus Sweet clover letent (?)	Arabis mosaic nepovirus	Alfalfa mosaic alfamovirus	Susceptible to:	
Sweet clover latent (?)	Asystasia gangetica mottle	Beet western yellows	Alfalfa mosaic alfamovirus	
nucleorhabdovirus	(?) potyvirus Brood boor wilt fobovirus	luteovirus	Bean distortion dwarf (?)	
Sweet clover necrotic	Broad bean wilt fabavirus	Chicory yellow mottle	bigeminivirus Boot wootorn vollowe	
nosaic dianthovirus	Carnation mottle	nepovirus Cucumbar magnia	Beet western yellows	
Tobacco etch potyvirus	carmovirus	Cucumber mosaic	luteovirus	
Tobacco rattle tobravirus	Carnation ringspot	cucumovirus	Cassia mild mosaic (?)	
Tobacco ringspot nepovirus	dianthovirus	Tobacco rattle tobravirus	carlavirus	
obacco streak ilarvirus	Cherry leaf roll nepovirus	Tobacco ringspot nepovirus	Celery latent (?) potyvirus	
Furnip mosaic potyvirus	Clover yellow vein	Tomato spotted wilt	Chilli veinal mottle (?)	
Watermelon mosaic 2	potyvirus	tospovirus	potyvirus	
ootyvirus	Cowpea mosaic comovirus	Turnip crinkle carmovirus	Chino del tomat,	
White clover mosaic	Cucumber mosaic	Turnip mosaic potyvirus	bigeminivirus	
ootexvirus	cucumovirus	Turnip yellow mosaic	Cucumber mosaic	
Frifolium dubium	Cymbidium ringspot	tymovirus	cucumovirus	
Synonyms:	tombusvirus	Coreopsis lanceolata	Datura distortion mosaic	
Trifolium filiforme var.	Dogwood mosaic (?)	Susceptible to:	potyvirus	
lubium; Trifolium minus;	nepovirus	Bidens mosaic potyvirus	Eggplant mosaic tymovirus	
Trifolium parviflorum; Trifolium	Elm mottle ilarvirus	Papaver rhoeas	Eggplant mottled dwarf	
procumbens	Groundnut eyespot	Common names:	nucleorhabdovirus	
Common names:	potyvirus	Corn poppy; Shirley poppy;	Eggplant severe mottle (?)	
	Maracuja mosaic (?)	Field poppy	potyvirus	
Small hop clover; Suckling	Maraeuja mosare (1)			
clover; Lesser yellow trefoil; Low	tobamovirus	Susceptible to:	Henbane mosaic potyvirus	

TABLE 4-continued

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	
clostrovirus	Melon Ourmia ourmiavirus	Pennisetum Alopecuroides	potyvirus	
Linum grandiflorum	Paprika mild mottle	Pennisetum Ruppelianum	Carnation mottle	
Synonyms:	tobamovirus	Pennisetum Alopecuroides	carmovirus	
Linum rubrum	Peanut stunt cucumovirus	Pennisetum Alopecuroides	Carrot mosaic (?) potyvirus	
Common names:	Pelargonium vein clearing (?)	Pennisetum Alopecuroides	Cassava green mottle	
Flowering flax	cytorhabdovirus	Pennisetum Setaceum	nepovirus	
Susceptible to:	Pepper hausteco	Pennisetum Setaceum	Cassia mild mosaic (?)	
Beet pseudo-yellows (?) closterovirus	bigeminivirus Pepper Indian mottle	Pennisetum Cassian Phalaris Arundinacea	carlavirus Chickpea chlorotic dwarf (?)	
Oat blue dwarf marafivirus	potyvirus	Phalaris Arundinacea	monogeminivirus	
Linum usitatissimum	Pepper mild mosaic (?)	Phalaris Arundinacea	Chino del tomat,	
Synonyms:	potyvirus	Sesleria Autumnalis	bigeminivirus	
Linum crepitans; Linum	Pepper mild mottle	Sesleria Caerulea	Clover wound tumor	
humile; Linum usitatissimum ssp.	tobamovirus	Sporobolus Helerolepsis	phytoreovirus	
transitorium; Linum usitatissimum	Pepper mild tigr, (?)	Stipa Capillata	Commelina X potexvirus	
var. humile	bigeminivirus	Stipa Extremiorientalis	Cowpea mild mottle (?)	
Common names:	Pepper Moroccan	Stipa Gigantea	carlavirus	
Flax; Linseed; Lino	tombusvirus	Stipa Tenuissima	Croton yellow vein mosaic	
Susceptible to:	Pepper mottle potyvirus	Stipa Grandis	bigeminivirus	
Alfalfa mosaic alfamovirus	Pepper ringspot tobravirus	Stipa Pennata	Cucumber mosaic	
Beet curly top	Pepper severe mosaic	Stipa Ucrainica	cucumovirus	
hybrigeminivirus	potyvirus	Impatiens	Cymbidium ringspot	
Beet pseudo-yellows (?)	Pepper Texas bigeminivirus	Impatiens necrotic spot tospovirus	tombusvirus	
closterovirus	Pepper veinal mottle	Carnation mottle carmovirus	Datura distortion mosaic	
Oat blue dwarf marafivirus	Potyvirus	Helenium S carlavirus	potyvirus	
Tobacco rattle tobravirus	Physalis mosaic tymovirus	Impatiens latent (?) potexvirus	Datura innoxia Hungarian	
ORNAMENTAL GRASSES	Pittosporum vein yellowing	Aster chlorotic stunt (?) carlavirus	mosaic (?) potyvirus	
Acorus Gramineus	nucleorhabdovirus	Dasheen mosaic potyvirus	Datura mosaic (?) potyvirus	
Acorus Calamus	Potato aucuba mosaic	Aglaonema	Datura necrosis potyvirus	
Acorus Gramineus	potexvirus	Alocasia	Datura yellow vein nucleorhabdovirus	
Alopecurus Pratensis	Potato mop-top furovirus Potato Y potyvirus	Amorphophallus Arisaema		
Andropogon Scoparius Andropogon Gerardii	Red pepper 1 (?)	Caladium hortulanum	Dogwood mosaic (?) nepovirus	
Anaropogon Geruran Arrhenatherum Elatius	alphacryptovirus	Chenopodium amaranticolor	Dulcamara mottle	
Arundo Formosana	Red pepper 2 (?)	Chenopodium ambrosioides	tymovirus	
Briza Media	alphacryptovirus	Chenopodium quinoa	Eggplant green mosaic	
Calamagrostis Acutiflora	Ribgrass mosaic	Colocasia esculenta	potyvirus	
Calamagrostis Arundinacea	tobamovirus	Cryptocoryne	Eggplant mosaic tymovirus	
Calamagrostis Acutiflora	Serrano golden mosaic	Cyrtosperma	Eggplant mottled dwarf	
Calamagrostis Acutiflora	bigeminivirus	Dieffenbachia picta	nucleorhabdovirus	
Carex Glauca	Sweet potato ringspot (?)	Nicotiana benthamiana	Eggplant severe mottle (?)	
Carex Siderostica	nepovirus	Philodendron selloum	potyvirus	
Carex Albula	Tobacco etch potyvirus	Philodendron verrucosum	Elderberry latent (?)	
Carex Nigra	Tobacco leaf curl	Richardia	carmovirus	
Carex Muskingumensis	bigeminivirus	Saponaria vaccaria	Elm mottle ilarvirus	
Carex Riparia	Tobacco mild green mosaic	Spathiphyllum	Epirus cherry ourmiavirus	
Carex Evergold	tobamovirus	Tetragonia tetragonioides	Foxtail mosaic potexvirus	
Carex Comans	Tobacco mosaic satellivirus	Xanthosoma caracu	Groundnut eyespot	
Cortaderia Selloana	Tobacco rattle tobravirus	Zantedeschia (no species name	potyvirus	
Cortaderia Selloana Rosea	Tobacco streak ilarvirus	provided)	Henbane mosaic potyvirus	
Deschampsia Cespitosa	Tomato bushy stunt	Zantedeschia elliottiana	Lettuce necrotic yellows	
Elymus Arenarius	tombusvirus	Colocasia bobone disease (?)	cytorhabdovirus	
Erianthus Ravennae	Tomato mosaic tobamovirus	rhabdovirus	Maracuja mosaic (?)	
Ovina Gigantea Ovina Clausa	Tomato Peru potyvirus	Dasheen bacilliform (?)	tobamovirus Mariaeld mettle petruirus	
Ovina Glauca Glucaria Marima	Tomato spotted wilt	badnavirus Dashaan masaia patunirus	Marigold mottle potyvirus	
Glyceria Maxima Hakonechloa Macra	tospovirus Lycopersicon esculentum	Dasheen mosaic potyvirus Colocasia esculenta	Melilotus mosaic (?) potyvirus	
Hakonechioa Macra Hakonechloa Macra	Common names:	Konjak mosaic (?) potyvirus	Melon Ourmia ourmiavirus	
Hakonecnioa Macra Helictotrichon Sempervirens	Tomato; Tomate	Philodendron	Nerine X potexvirus	
Heucionicnon sempervirens Holcus Variegated	Susceptible to:	oxvcardium	Okra leaf-curl bigeminivirus	
Hystrix Patula	Abelia latent tymovirus	Philodendron selloum	Ononis yellow mosaic	
Imperata Red Baron	Abutilon mosaic	Abelia latent tymovirus	tymovirus	
Juncus Effusus	bigeminivirus	Abelia grandiflora	Parietaria mottle ilarvirus	
Juncus Ensifolius	Alfalfa mosaic alfamovirus	Abelmoschus esculentus	Parsnip yellow fleck	
Juncus Filiformis	Arabis mosaic nepovirus	Acer palmatum	sequivirus	
Juncus Inflexus	Arracacha A nepovirus	Amaranthus caudatus	Pea streak carlavirus	
Koeleria Cristata	Arracacha B (?) nepovirus	Atropa belladonna	Peanut clump furovirus	
Koeleria Glauca	Beet curly top	Brassica campestris ssp.	Peanut stunt cucumovirus	
Luzula Sylvatica	hybrigeminivirus	pekinensis	Pelargonium line pattern (?)	
Melica Ćiliata	Beet western yellows	Catharanthus roseus	carmovirus	
Melica Nutans	luteovirus	Celosia argentea	Pelargonium zonate spot	
Miscanthus Sinensis	Blueberry leaf mottle	Chenopodium amaranticolor	ourmiavirus	
Miscanthus Sinensis Molinia Caerulea	Blueberry leaf mottle nepovirus Brinjal mild mosaic (?)	Chenopodium amaranticolor Chenopodium murale	ourmiavirus Pepino mosaic potexvirus	

TABLE 4-continued

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued	
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name
Datura metel	potyvirus	Amaranthus caudatus ssp.	Tobacco vein-distorting (?)
Datura stramonium	Pepper mild tigr, (?)	mantegazzianus; Amaranthus	luteovirus
Glycine max	bigeminivirus	caudatus var. alopecurus;	Tobacco vein mottling
Gomphrena globosa	Pepper Moroccan	Amaranthus dussii; Amaranthus	potyvirus
Gossypium hirsutum	tombusvirus Baaran and the antennion	edulis; Amaranthus	Tobacco yellow dwarf
Hordeum vulgare	Pepper mottle potyvirus	mantegazzianus	monogeminivirus
obelia erinus sycopersicon esculentum	Pepper ringspot tobravirus Pepper severe mosaic	Common names: Inca wheat; Love-lies-	Tobacco yellow net (?) luteovirus
Momordica balsamina	potyvirus	bleeding; Tassel-flower; Kiwichi;	Tobacco yellow vein
Vicotiana clevelandii	Pepper Texas bigeminivirus	Coimi	assistor (?) luteovirus
Vicotiana glutinosa	Pepper veinal mottle	Susceptible to:	Tobacco yellow vein (?)
Vicotiana rustica	potyvirus	Abelia latent tymovirus	umbravirus
Petunia x hybrida	Physalis mosaic tymovirus	Alfalfa mosaic alfamovirus	Tomato aspermy
Physalis peruviana	Pittosporum vein vellowing	Amaranthus leaf mottle	cucumovirus
esamum indicum	nucleorhabdovirus	potyvirus	Tomato Australian leafcurl
Solanum melongena	Plantain X potexvirus	Amaranthus mosaic (?)	bigeminivirus
olanum tuberosum	Plum pox potyvirus	potyvirus	Tomato black ring
etragonia tetragonioides	Potato 14R (?) tobamovirus	Arracacha A nepovirus	nepovirus
ïthonia speciosa	Potato Andean latent	Arracacha B (?) nepovirus	Tomato bushy stunt
'orenia fournieri	tymovirus	Bean yellow mosaic	tombusvirus
'icia faba	Potato Andean mottle	potyvirus	Tomato chlorotic spot (?)
Allium	comovirus	Beet curly top	tospovirus
usceptible to:	Potato aucuba mosaic	hybrigeminivirus	Tomato golden mosaic
Dnion yellow dwarf	potexvirus	Beet mosaic potyvirus	bigeminivirus
ootyvirus	Potato black ringspot	Cactus X potexvirus	Tomato infectious chlorosis (?)
Allium ampeloprasum var.	nepovirus	Carnation mottle	closterovirus
olmense	Potato leafroll luteovirus	carmovirus	Tomato mild mottle (?)
Farlic common latent (?)	Potato M carlavirus	Carnation ringspot	potyvirus To conta and taken a
arlavirus	Potato mop-top furovirus	dianthovirus	Tomato mosaic tobamovirus
llium ampeloprasum var.	Potato U nepovirus	Carnation vein mottle potyvirus	Tomato mottle
<i>ectivum</i> Susceptible to:	Potato V potyvirus Potato Y potyvirus	Celery latent (?) potyvirus	bigeminivirus Tomato Peru potyvirus
Sint-Jan's onion latent (?)	Potato yellow mosaic	Chicory yellow mottle	Tomato pseudo curly top (?)
arlavirus	bigeminivirus	nepovirus	hybrigeminivirus
Allium cepa	Raspberry ringspot	Clover yellow mosaic	Tomato ringspot nepovirus
Synonyms:	nepovirus	potexvirus	Tomato spotted wilt
Allium ascalonicum; Allium	Red clover necrotic mosaic	Clover yellow vein	tospovirus
epa var. aggregatum; Allium	dianthovirus	potyvirus	Tomato top necrosis (?)
epa var. solaninum	Ribgrass mosaic	Cucumber mosaic	nepovirus
Common names:	tobamovirus	cucumovirus	Tomato vein clearing
Dnion; Shallot; Tama-negi;	Rose (?) tobamovirus	Cymbidium ringspot	nucleorhabdovirus
Schalot; Potato onion; Multiplier	Rubus Chinese seed-borne (?)	tombusvirus	Tomato yellow leaf curl
nion; Cebolla; Spanish onion	nepovirus	Dahlia mosaic caulimovirus	bigeminivirus
Susceptible to:	Serrano golden mosaic	Elderberry carlavirus	Tomato yellow mosaic
eek yellow stripe potyvirus	bigeminivirus	Grapevine fanleaf nepovirus	bigeminivirus
Dnion mite-borne latent (?)	Solanum apical leaf curling (?)	Heracleum latent trichovirus	Tulip chlorotic blotch
otexvirus	bigeminivirus	Humulus japonicus ilarvirus	potyvirus
Dnion yellow dwarf	Soybean crinkle leaf (?)	Iris fulva mosaic potyvirus	Tulip X potexvirus
otyvirus	bigeminivirus	Lamium mild mottle	Turnip crinkle carmovirus
epper venial mottle	Soybean mild mosaic virus	fabavirus	Ullucus mild mottle
otyvirus	Strawberry latent ringspot (?)	Lettuce mosaic potyvirus	tobamovirus
hallot latent carlavirus	nepovirus	Maclura mosaic	White clover mosaic
hallot mite-borne latent (?)	Sunflower ringspot (?)	macluravirus	potexvirus
otexvirus	ilarvirus	Marigold mottle potyvirus	Wild potato mosaic
hallot yellow stripe (?)	Sweet potato mild mottle	Peanut stunt cucumovirus	potyvirus Wincharry latert virus
otyvirus	ipomovirus Temorillo mossio potuvirus	Plantain X potexvirus	Wineberry latent virus
int-Jan's onion latent (?) arlavirus	Tamarillo mosaic potyvirus	Potato 14R (?) tobamovirus	Nicotiana benthamiana Susceptible to:
arlavirus obacco rattle tobravirus	Tamus latent (?) potexvirus Tobacco etch potyvirus	Potato Andean latent tymovirus	Susceptible to: Ahlum waterborne (?)
Velsh onion yellow stripe (?)	Tobacco leaf curl	Potato black ringspot	carmovirus
otyvirus	bigeminivirus	nepovirus	Alstroemeria (?) ilarvirus
amaranthaceae	Tobacco mild green mosaic	Potato leafroll luteovirus	Alstroemeria mosaic
usceptible to:	tobamovirus	Red clover necrotic mosaic	potyvirus
Apple stem grooving	Tobacco mosaic satellivirus	dianthovirus	Alstroemeria streak (?)
apillovirus	Tobacco mosaic	Ribgrass mosaic	potyvirus
nsusceptible to:	tobamovirus	tobamovirus	Amazon lily mosaic (?)
Joandzeia necrotic mosaic	Tobacco mottle umbravirus	Telfairia mosaic potyvirus	potyvirus
ymovirus	Tobacco necrosis necrovirus	Tobacco etch potyvirus	Apple mosaic ilarvirus
Amaranthus bicolor	Tobacco necrotic dwarf	Tobacco necrosis necrovirus	Arracacha Y potyvirus
nsusceptible to:	luteovirus	Tobacco rattle tobravirus	Artichoke latent potyvirus
	Tobacco rattle tobravirus	Tobacco ringspot nepovirus	Artichoke latent S (?)
		impopor nopornum	
		Tobacco streak ilarvirus	carlavirus
Onion mite-borne latent (?) potexvirus Amaranthus caudatus	Tobacco ringspot nepovirus Tobacco streak ilarvirus	Tobacco streak ilarvirus Tomato black ring	carlavirus Artichoke mottled crinkle

TABLE 4-continued

Plant or Virus Name Tomato spotted wilt tospovirus Turnip mosaic potyvirus Ullucus mild mottle tobamovirus Viola mottle potexvirus Watermelon mosaic 2 potyvirus Zygocactus Montana X (?) potexvirus Amaranthus tricolor Synonyms: Amaranthus gangeticus; Amaranthus gangeticus var. melancholicus; Amaranthus mangostanus; Amaranthus polygamus; Amaranthus tricolor ssp. mangostanus; Amaranthus tricolor ssp. tristis Common names: Chinese amaranth; Tampala; Ganges amaranth Susceptible to: Amaranthus leaf mottle potyvirus Amaranthus mosaic (?) potyvirus Apple mosaic ilarvirus Amaryllis Susceptible to: Amaryllis (?) alphacryptovirus Narcissus Susceptible to: Narcissus yellow stripe potyvirus Insusceptible to: Silene X (?) potexvirus Narcissus jonquilla Common names: Jonguil Susceptible to: Strawberry latent ringspot (?) nepovirus Insusceptible to: Ornithogalum mosaic potyvirus Narcissus poeticus Common names: Narcissus; Pheasant's-eye; Poet's narcissus Susceptible to: Narcissus tip necrosis (?) carmovirus Narcissus pseudonarcissus Common names: Daffodil: Common daffodil Susceptible to: Arabis mosaic nepovirus Narcissus late season yellows (?) potyvirus Narcissus latent macluravirus Narcissus mosaic potexvirus Narcissus tip necrosis (?) carmovirus Raspberry ringspot nepovirus Tobacco rattle tobravirus Tomato black ring nepovirus Yucca Susceptible to: Furcraea necrotic streak (?)

Plant or Virus Name Artichoke vein banding (?) nepovirus Asparagus 3 potexvirus Asystasia gangetica mottle (?) potyvirus Barley yellow streak mosaic virus Bean calico mosaic bigeminivirus Bean common mosaic potyvirus Beet curly top hybrigeminivirus Blueberry leaf mottle nepovirus Blueberry necrotic shock ilarvirus Caper latent carlavirus Caraway latent (?) nepovirus Carrot mottle mimic umbravirus Carrot mottle umbravirus Carrot yellow leaf (?) closterovirus Cassava African mosaic bigeminivirus Cassava brown streakassociated (?) carlavirus Cassava brown streak potyvirus Cassava Caribbean mosaic (?) potexvirus Cassava Colombian symptomless (?) potexvirus Cassava common mosaic (?) potexvirus . Cassava green mottle nepovirus Cassava Indian mosaic bigeminivirus Cassava Ivorian bacilliform ourmiavirus Cassava X potexvirus Cherry leaf roll nepovirus Chickpea bushy dwarf potyvirus Chickpea chlorotic dwarf (?) monogeminivirus Chickpea distortion mosaic potyvirus Chicory yellow mottle nepovirus Chino del tomat, bigeminivirus Citrus ringspot virus Cowpea chlorotic mottle bromovirus Croton yellow vein mosaic bigeminivirus Cucumber necrosis tombusvirus Cymbidium ringspot tombusvirus Cynara (?) nucleorhabdovirus Dandelion yellow mosaic seauivirus Dasheen mosaic potyvirus Desmodium mosaic potyvirus Dioscorea green banding mosaic potyvirus Dioscorea latent (?)

dianthovirus

Ribbon plant

potexvirus

potexvirus

carlavirus

potyvirus

Synonyms:

Susceptible to:

Beet curly top

tymovirus

tymovirus

carmovirus

nepovirus

potexvirus

comovirus

nepovirus

tymovirus

potexvirus

potyvirus

potyvirus

ilarvirus

nepovirus

cucumovirus

TABLE 4-continued

Plant or Virus Name Plant or Virus Name potexvirus Chlorophytum comosum Dogwood mosaic (?) nepovirus Common names: Spider plant; Spider-ivy; Eggplant green mosaic potyvirus Insusceptible to: Eggplant mottled dwarf Onion mite-borne latent (?) nucleorhabdovirus Eggplant severe mottle (?) Shallot mite-borne latent (?) potyvirus Elderberry latent (?) Sint-Jan's onion latent (?) carmovirus Epirus cherry ourmiavirus Tradescantia-Zebrina Euphorbia mosaic bigeminivirus Grapevine A (?) trichovirus Grapevine Algerian latent Catharanthus roseus Ammocallis rosea; tombusvirus Lochnera rosea; Vinca rosea Grapevine Bulgarian latent Common names: nepovirus Bright-eyes; Madagascar Grapevine chrome mosaic periwinkle; Old-maid; Rose nepovirus periwinkle; Rosy periwinkle Grapevine fanleaf nepovirus Groundnut chlorotic spot (?) Abelia latent tymovirus potexvirus Alfalfa mosaic alfamovirus Groundnut rosette Apple mosaic ilarvirus umbravirus Hibiscus latent ringspot Bean pod mottle comovirus nepovirus hybrigeminivirus Belladonna mottle Hydrangea mosaic ilarvirus Ivy vein clearing (?) cytorhabdovirus Cacao yellow mosaic Kalanchoe isometric virus Lato River tombusvirus Carnation mottle Lettuce big-vein varicosavirus Cassava green mottle Lettuce mosaic potyvirus Lilac chlorotic leafspot Cherry leaf roll nepovirus capillovirus Citrus leaf rugose ilarvirus Lily X potexvirus Citrus ringspot virus Lucerne Australian Clover wound tumor symptomless (?) nepovirus phytoreovirus Clover yellow mosaic Maracuja mosaic (?) tobamovirus Melon Ourmia ourmiavirus Melothria mottle (?) Cowpea severe mosaic potyvirus Cucumber mosaic Nandina mosaic (?) potexvirus Dogwood mosaic (?) Narcissus latent macluravirus Dulcamara mottle Narcissus tip necrosis (?) carmovirus Elm mottle ilarvirus Neckar River tombusvirus Erysimum latent tymovirus Nerine potyvirus Nicotiana velutina mosaic (?) Foxtail mosaic potexvirus Humulus japonicus ilarvirus furovirus Lilac ring mottle ilarvirus Nandina mosaic (?) Oat golden stripe furovirus Okra mosaic tymovirus Olive latent 1 (?) Narcissus mosaic potexvirus sobemovirus Okra mosaic tymovirus Olive latent 2 (?) Pea seed-borne mosaic ourmiavirus Paprika mild mottle Peach enation (?) nepovirus tobamovirus Peanut stunt cucumovirus Parsnip yellow fleck Pepper ringspot tobravirus sequivirus Pepper veinal mottle Passiflora ringspot potyvirus Plum American line pattern Peanut chlorotic streak caulimovirus Peanut clump furovirus Poplar mosaic carlavirus Potato 14R (?) tobamovirus Peanut green mosaic Potato black ringspot potyvirus Peanut yellow spot

TABLE 4-continued

Plant or Virus Name Potato T trichovirus Prune dwarf ilarvirus Prunus necrotic ringspot ilarvirus Scrophularia mottle tymovirus Spring beauty latent bromovirus Tobacco mosaic satellivirus Tobacco necrosis necrovirus Tobacco rattle tobravirus Tobacco ringspot nepovirus Tobacco streak ilarvirus Tobacco stunt varicosavirus Tomato spotted wilt tospovirus Tulare apple mosaic ilarvirus Turnip crinkle carmovirus Watermelon mosaic 2 potyvirus Wild cucumber mosaic tymovirus . Hedera helix Common names: English ivy Susceptible to: Ivy vein clearing (?) cytorhabdovirus sparagus officinalis Synonyms: Asparagus longifolius Common names: Garden asparagus; Asparagus; Esparrag Susceptible to: Arabis mosaic nepovirus Asparagus 1 potyvirus Asparagus 2 ilarvirus Strawberry latent ringspot (?) nepovirus Tobacco streak ilarvirus Dryopteris filix-mas Common names: Male fern Susceptible to: Fern (?) potyvirus Polystichum falcatum Susceptible to: Harts tongue fern (?) tobravirus Phyllitis scolopendrium Synonyms: Asplenium scolopendrium Common names: Hart's-tongue fern Susceptible to: Harts tongue fern (?) tobravirus Aucuba japonica Synonyms: Aucuba japonica var. variegata Common names: Spotted-laurel; Japaneselaurel Susceptible to: Aucuba ringspot (?) badnavirus Cycas necrotic stunt nepovirus Begonia elatior Susceptible to: Carnation mottle

Plant or Virus Name tospovirus Pelargonium vein clearing (?) cytorhabdovirus Pepper Moroccan tombusvirus Pepper mottle potyvirus Pepper ringspot tobravirus Pepper Texas bigeminivirus Pepper veinal mottle potyvirus Physalis mosaic tymovirus Pittosporum vein yellowing nucleorhabdovirus Plantain 6 (?) carmovirus Plantain 7 (?) potyvirus Plantain X potexvirus Plum American line pattern ilarvirus Plum pox potyvirus Poinsettia mosaic (?) tymovirus Potato 14R (?) tobamovirus Potato Andean latent tymovirus Potato Andean mottle comovirus Potato black ringspot nepovirus Potato mop-top furovirus Potato T trichovirus Prune dwarf ilarvirus Prunus necrotic ringspot ilarvirus Red clover necrotic mosaic dianthovirus Rice stripe necrosis (?) furovirus Rubus Chinese seed-borne (?) nepovirus Silene X (?) potexvirus Sitke waterborne (?) tombusvirus Solanum apical leaf curling (?) bigeminivirus Solanum nodiflorum mottle sobemovirus Sonchus yellow net nucleorhabdovirus Soybean mosaic potyvirus Sweet potato feathery mottle potyvirus Sweet potato latent (?) potyvirus Sweet potato mild mottle ipomovirus Sweet potato ringspot (?) nepovirus Sweet potato sunken vein (?) closterovirus Tamus latent (?) potexvirus Telfairia mosaic potyvirus Tobacco mosaic satellivirus Tobacco mosaic tobamovirus Tobacco rattle tobravirus Tobacco streak ilarvirus Tobacco stunt varicosavirus Tomato Australian leafcurl bigeminivirus Tomato bushy stunt tombusvirus Tomato golden mosaic bigeminivirus Tomato mild mottle (?)

Begonia x tuberhybrida Common names: Hybris tuberous begonia Insusceptible to: Aster chlorotic stunt (?) carlavirus Catalpa bignonioides Synonyms Catalpa bignonioides f. aurea Common names: Catawba; Common catalpa; Indian-bean; Southern catalpa; Cigartree; Smoking-bean Susceptible to: Scrophularia mottle tymovirus Ácer palmatum Abelia latent tymovirus Betula Susceptible to: Cherry leaf roll nepovirus Ceiba pentandra Synonyms: Bombax pentandrum; Ceiba casearia; Eriodendron anfractuosum Common names: Ceiba; Kapok; Silk-cottontree; White silk-cotton-tree; Kapokbaum; Kapokier; Arbekapok Susceptible to: Cacao swollen shoot badnavirus Cacao yellow mosaic tymovirus Okra mosaic tymovirus Myosotis sylvatica Synonyms: Myosotis alpestris; Myosotis oblongata Common names: Garden forget-me-not; Wood forget-me-not Susceptible to: Arabis mosaic nepovirus Carnation ringspot dianthovirus Cymbidium ringspot tombusvirus Tobacco rattle tobravirus Tobacco ringspot nepovirus Tomato black ring nepovirus Ananas comosus Synonyms: Ananas duckei; Ananas sativus; Ananas sativus var. duckei; Bromelia ananas; Bromelia comosa Common names: Pineapple; Pina Susceptible to: Pineapple chlorotic leaf streak (?) nucleorhabdovirus Pineapple wilt-associated (?) closterovirus Tomato spotted wilt tospovirus Buxus sempervirens Synonyms: Buxus colchica

TABLE 4-continued

Plant or Virus Name potyvirus Tomato mosaic tobamovirus Tomato mottle bigeminivirus Tomato ringspot nepovirus Tomato yellow leaf curl bigeminivirus Tomato yellow mosaic bigeminivirus Tropaeolum 1 potyvirus Tropaeolum 2 potyvirus Tulip chlorotic blotch potyvirus Tulip halo necrosis (?) virus Tulip X potexvirus Ullucus mild mottle tobamovirus Ullucus mosaic potyvirus Vanilla necrosis potyvirus Watercress yellow spot virus Watermelon mosaic 2 potyvirus Weddel waterborne (?) carmovirus Wild potato mosaic potyvirus Yam mosaic potyvirus Nicotiana tabacum Synonyms: Nicotiana chinensis; Nicotiana tabacum var. macrophylla Common names: Tobacco Susceptible to: Abutilon mosaic bigeminivirus Alfalfa mosaic alfamovirus Alstroemeria (?) ilarvirus Alstroemeria mosaic potyvirus Amaranthus leaf mottle potyvirus Arabis mosaic nepovirus Arracacha A nepovirus Arracacha B (?) nepovirus Arracacha Y potyvirus Artichoke Italian latent nepovirus Artichoke yellow ringspot nepovirus Asparagus 2 ilarvirus Asparagus 3 potexvirus Asystasia gangetica mottle (?) potyvirus Barley stripe mosaic hordeivirus Bean distortion dwarf (?) bigeminivirus Bean yellow mosaic potyvirus Beet curly top hybrigeminivirus Beet pseudo-yellows (?) closterovirus Belladonna mottle tymovirus Bidens mosaic potyvirus Blueberry leaf mottle nepovirus Blueberry necrotic shock ilarvirus Bramble yellow mosaic (?)

Plant or Virus Name

carmovirus

TABLE 4-continued		TABLE 4-continued	
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name
Common names:	potyvirus	posoposa; Papaya carica	Eggplant mottled dwarf
Boxwood; Common	Broad bean wilt fabavirus	Common names:	nucleorhabdovirus
boxwood; Turkish boxwood	Burdock yellow mosaic (?)	Papaya; Pawpaw	Eggplant severe mottle (?)
Susceptible to:	potexvirus	Susceptible to:	potyvirus
Arabis mosaic nepovirus	Cacao necrosis nepovirus	Croton yellow vein mosaic	Elderberry latent (?)
Cactaceae family	Cacao yellow mosaic	bigeminivirus Bonous, mossis, notomuinus	carmovirus Elme mattla ileminus
Including: Austrocylindropuntia cylindrica	tymovirus Carnation ringspot	Papaya mosaic potexvirus Papaya ringspot potyvirus	Elm mottle ilarvirus Epirus cherry ourmiavirus
Cactaceae	dianthovirus	Watermelon mosaic 1	Eucharis mottle (?)
Carnegiea gigantea (syn. Cereus	Cassava African mosaic	potyvirus	nepovirus
giganteus)	bigeminivirus	Dianthus barbatus	Foxtail mosaic potexvirus
Saguaro; Giant cactus	Cassava green mottle	Common names:	Frangipani mosaic
Cereus	nepovirus	Sweet William	tobamovirus
Chamaecereus sylvestrii	Cassava Indian mosaic	Susceptible to:	Galinsoga mosaic
Echinocereus procumbens	bigeminivirus	Alfalfa mosaic alfamovirus	carmovirus
Echinopsis	Cassava Ivorian bacilliform	Arabis mosaic nepovirus	Grapevine Bulgarian latent
Epiphyllum	ourmiavirus	Beet curly top	nepovirus
Ferocactus acanthodes (syn.	Cassia mild mosaic (?)	hybrigeminivirus	Grapevine chrome mosaic
Echinocactus acanthodes)	carlavirus	Beet mosaic potyvirus	nepovirus
Opuntia engelmannii	Cassia severe mosaic (?)	Carnation latent carlavirus Carnation mottle	Grapevine fanleaf nepovirus
Opuntia vulgaris (syn. Cactus monacanthos; Opuntia	closterovirus	carmovirus	Guar top necrosis virus
monacantha)	Celery latent (?) potyvirus Cherry leaf roll nepovirus	Carnation necrotic fleck	Henbane mosaic potyvirus Hibiscus latent ringspot
Prickly-pear cactus; Tuna;	Chickpea chlorotic dwarf (?)	closterovirus	nepovirus
Prickly-pear; Drooping prickly-	monogeminivirus	Carnation (?) rhabdovirus	Hippeastrum mosaic
pear	Chicory yellow mottle	Carnation ringspot	potyvirus
Pereskia saccharosa	nepovirus	dianthovirus	Hop American latent
Schlumbergera bridgesii	Chilli veinal mottle (?)	Carnation vein mottle	carlavirus
Zygocactus	potyvirus	potyvirus	Humulus japonicus ilarvirus
Zygocactus truncatus	Chino del tomat,	Carnation yellow stripe (?)	Ivy vein clearing (?)
Zygocactus x Schlumbergera	bigeminivirus	necrovirus	cytorhabdovirus
Susceptible to:	Citrus ringspot virus	Clover wound tumor	Kalanchoe isometric virus
Cactus X potexvirus	Clover wound tumor	phytoreovirus	Kyuri green mottle mosaic
Cactus 2 carlavirus	phytoreovirus	Melon Ourmia ourmiavirus	tobamovirus
Lobelia erinus	Clover yellow vein	Okra mosaic tymovirus	Lamium mild mottle
Common names:	potyvirus	Peanut stunt cucumovirus	fabavirus
Edging lobelia	Commelina X potexvirus	Pelargonium line pattern (?)	Lilac chlorotic leafspot
Susceptible to: Abelia latent tymovirus	Cowpea chlorotic mottle bromovirus	carmovirus Poteto blogh ringenot	capillovirus Lilac ring mottle ilarvirus
Arabis mosaic nepovirus	Cowpea mosaic comovirus	Potato black ringspot nepovirus	Lisianthus necrosis (?)
Carnation ringspot	Cowpea mottle (?)	Potato M carlavirus	necrovirus
dianthovirus	carmovirus	Silene X (?) potexvirus	Lucerne Australian latent
Cherry leaf roll nepovirus	Cowpea severe mosaic	Strawberry latent ringspot (?)	nepovirus
Elm mottle ilarvirus	comovirus	(?) nepovirus	Lucerne Australian
Peanut stunt cucumovirus	Croton yellow vein mosaic	Tobacco ringspot nepovirus	symptomless (?) nepovirus
Strawberry latent ringspot	bigeminivirus	Tomato bushy stunt	Lucerne transient streak
(?) nepovirus	Cucumber green mottle	tombusvirus	sobemovirus
Tobacco rattle tobravirus	mosaic tobamovirus	Viola mottle potexvirus	Lychnis ringspot
Tomato black ring	Cucumber mosaic	Dianthus caryophyllus	hordeivirus
nepovirus	cucumovirus	Common names:	Maclura mosaic
Humulus japonicus	Cucumber necrosis	Carnation, Clavel	macluravirus
Synonyms:	tombusvirus	Susceptible to:	Maracuja mosaic (?)
<i>Humulus scandens</i> Common names:	Cymbidium ringspot tombusvirus	Alfalfa mosaic alfamovirus Arabis mosaic nepovirus	tobamovirus Marigold mottle potyvirus
Japanese hop	Datura Colombian potyvirus	Beet curly top	Melandrium yellow fleck
Susceptible to:	Datura distortion mosaic	hybrigeminivirus	bromovirus
Hop latent carlavirus	potyvirus	Carnation 1	Melilotus mosaic (?)
Humulus japonicus ilarvirus	Datura innoxia Hungarian	alphacryptovirus	potyvirus
Lonicera	mosaic (?) potyvirus	Carnation 2 (?)	Melon Ourmia ourmiavirus
Susceptible to:	Datura mosaic (?) potyvirus	alphacryptovirus	Milk vetch dwarf nanavirus
Eggplant mottled dwarf	Datura necrosis potyvirus	Carnation etched ring	Myrobalan latent ringspot
nucleorhabdovirus	Datura shoestring potyvirus	caulimovirus	nepovirus
Pittosporum vein yellowing	Datura yellow vein	Carnation Italian ringspot	Narcissus latent
nucleorhabdovirus	nucleorhabdovirus	tombusvirus	macluravirus
Insusceptible to:	Dioscorea latent (?)	Carnation latent carlavirus	Neckar River tombusvirus
Tomato yellow leaf curl	potexvirus	Carnation mottle	Nerine potyvirus
bigeminivirus	Dogwood mosaic (?)	carmovirus	Nicotiana velutina mosaic (?)
Lonicera americana Succeptible tec	nepovirus	Carnation necrotic fleck	furovirus Odontoglossum ringenet
Susceptible to:	Eggplant green mosaic	Closterovirus	Odontoglossum ringspot
Honovenal-lo lotort	notraine		
Honeysuckle latent	potyvirus Eggplant mild mottle (2)	Carnation (?) rhabdovirus	tobamovirus Okra leaf-curl bigeminivirus
carlavirus	Eggplant mild mottle (?)	Carnation ringspot	Okra leaf-curl bigeminivirus

TABLE 4-continued

Raspberry ringspot

TABLE 4-continued		TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	
Carnation yellow stripe (?)	ourmiavirus	Cacao necrosis nepovirus	nepovirus	
necrovirus	Orchid fleck (?) rhabdovirus	Cacao yellow mosaic	Red clover necrotic mosaic	
Lettuce infectious yellows	Paprika mild mottle	tymovirus	dianthovirus	
(?) closterovirus	tobamovirus	Cactus X potexvirus	Red clover vein mosaic	
Melandrium yellow fleck	Parietaria mottle ilarvirus	Caraway latent (?)	carlavirus	
oromovirus	Parsnip yellow fleck	nepovirus	Rhynchosia mosaic	
Potato M carlavirus	sequivirus	Carnation latent carlavirus	bigeminivirus	
Fobacco stunt varicosavirus	Passionfruit woodiness	Carnation mottle	Ribgrass mosaic	
Gypsophila elegans	potyvirus	carmovirus	tobamovirus	
Common names:	Patchouli mosaic potyvirus	Carnation vein mottle	Rose (?) tobamovirus	
Baby's-breath	Pea early browning	potyvirus	Rubus Chinese seed-borne	
Susceptible to:	tobravirus	Celery latent (?) potyvirus	(?) nepovirus	
Belladonna mottle	Pea mosaic potyvirus	Cherry leaf roll nepovirus	Silene X (?) potexvirus	
ymovirus	Pea streak carlavirus	Chickpea chlorotic dwarf	Solanum nodiflorum mottle	
Lychnis ringspot	Peach enation (?) nepovirus	(?) monogeminivirus	sobemovirus	
nordeivirus	Peach rosette mosaic	Chicory yellow blotch (?)	Sonchus cytorhabdovirus	
Fobacco etch potyvirus	nepovirus	carlavirus	Sowbane mosaic	
Fobacco necrosis necrovirus	Peanut chlorotic streak	Clover yellow mosaic	sobemovirus	
Tobacco rattle tobravirus	caulimovirus	potexvirus	Soybean crinkle leaf (?)	
Fobacco ringspot nepovirus	Peanut clump furovirus	Clover yellow vein	bigeminivirus	
Fomato bushy stunt	Peanut stunt cucumovirus	potyvirus	Soybean mild mosaic virus	
ombusvirus	Pelargonium line pattern (?)	Cowpea chlorotic mottle	Soybean mosaic potyvirus	
Euonymus europaeus	carmovirus	bromovirus	Spinach latent ilarvirus	
Synonyms:	Pelargonium vein clearing	Cowpea mild mottle (?)	Strawberry latent ringspot (?)	
Euonymus vulgaris	(?) cytorhabdovirus	carlavirus	nepovirus	
Common names:	Pelargonium zonate spot	Croton yellow vein mosaic	Sunn-hemp mosaic	
European spindletree;	ourmiavirus	bigeminivirus	tobamovirus	
Spindletree	Pepino mosaic potexvirus	Cucumber mosaic	Sweet clover necrotic	
Susceptible to:	Pepper Indian mottle	cucumovirus	mosaic dianthovirus	
Arabis mosaic nepovirus	potyvirus	Cucumber soil-borne	Sweet potato latent (?)	
Strawberry latent ringspot (?)	Pepper mild mosaic (?)	carmovirus	potyvirus	
nepovirus	potyvirus	Cycas necrotic stunt	Sweet potato mild mottle	
Euonymus japonica	Pepper mild mottle	nepovirus	ipomovirus	
Susceptible to:	tobamovirus	Cymbidium ringspot	Sweet potato ringspot (?)	
Euonymus fasciation (?)	Pepper Moroccan	tombusvirus	nepovirus	
rhabdovirus	tombusvirus	Dogwood mosaic (?)	Tamarillo mosaic potyvirus	
Euonymus (?) rhabdovirus	Pepper mottle potyvirus	nepovirus	Telfairia mosaic potyvirus	
Beta vulgaris	Pepper ringspot tobravirus	Elderberry carlavirus	Tobacco etch potyvirus	
Common names:			Tobacco leaf curl	
Beet	Pepper severe mosaic potyvirus	Elderberry latent (?) carmovirus		
			bigeminivirus	
Susceptible to:	Pepper Texas bigeminivirus	Elm mottle ilarvirus	Tobacco mild green mosaic	
Alfalfa mosaic alfamovirus	Pepper veinal mottle	Epirus cherry ourmiavirus	tobamovirus	
Arabis mosaic nepovirus	potyvirus	Foxtail mosaic potexvirus	Tobacco mosaic satellivirus	
Arracacha A nepovirus	Physalis mosaic tymovirus	Grapevine Bulgarian latent	Tobacco mosaic	
Asparagus 2 ilarvirus	Pittosporum vein yellowing	nepovirus	tobamovirus	
Asparagus 3 potexvirus	nucleorhabdovirus	Grapevine fanleaf nepovirus	Tobacco mottle umbravirus	
Barley stripe mosaic	Plantain X potexvirus	Groundnut eyespot	Tobacco necrosis necrovirus	
nordeivirus	Plum American line pattern	potyvirus	Tobacco necrosis	
Beet 1 alphacryptovirus	ilarvirus	Helenium S carlavirus	satellivirus	
Beet 2 alphacryptovirus	Plum pox potyvirus	Heracleum latent trichovirus	Tobacco necrotic dwarf	
Beet 3 alphacryptovirus	Poinsettia mosaic (?)	Humulus japonicus ilarvirus	luteovirus	
Beet curly top	tymovirus	Impatiens latent (?)	Tobacco rattle tobravirus	
nybrigeminivirus	Poplar mosaic carlavirus	potexvirus	Tobacco ringspot nepovirus	
Beet distortion mosaic virus	Potato 14R (?) tobamovirus	Lettuce infectious yellows	Tobacco streak ilarvirus	
Beet leaf curl (?)	Potato A potyvirus	(?) closterovirus	Tobacco stunt varicosavirus	
habdovirus	Potato Andean mottle	Lettuce mosaic potyvirus	Tobacco vein-distorting (?)	
Beet mild yellowing	comovirus	Lettuce speckles mottle	luteovirus	
uteovirus	Potato aucuba mosaic	umbravirus	Tobacco vein mottling	
Beet mosaic potyvirus	potexvirus	Lilac chlorotic leafspot	potyvirus	
Beet necrotic yellow vein	Potato black ringspot	capillovirus	Tobacco wilt potyvirus	
urovirus	nepovirus	Marigold mottle potyvirus	Tobacco yellow dwarf	
Beet pseudo-yellows (?)	Potato mop-top furovirus	Mulberry latent carlavirus	monogeminivirus	
closterovirus	Potato T trichovirus	Odontoglossum ringspot	Tobacco yellow net (?)	
Beet soil-borne furovirus	Potato U nepovirus	tobamovirus	luteovirus	
Beet western yellows	Potato V potyvirus	Parsnip leafcurl virus	Tobacco yellow vein	
uteovirus	Potato X potexvirus	Parsnip yellow fleck	assistor (?) luteovirus	
Beet yellow net (?)	Potato Y potyvirus	sequivirus	Tobacco yellow vein (?)	
uteovirus	Potato yellow dwarf	Pea seed-borne mosaic	umbravirus	
Beet yellow stunt	nucleorhabdovirus	potyvirus	Tomato aspermy	
closterovirus	Primula mosaic potyvirus	Peanut clump furovirus	cucumovirus	
Beet yellows closterovirus	Primula mosaic potyvirus Primula mottle (?) potyvirus	Peanut stunt cucumovirus	Tomato Australian leafcurl	
	Primula mottle (?) potyvirus Prune dwarf ilarvirus			
Broad bean wilt fabavirus		Pelargonium line pattern (?)	bigeminivirus Tomato black ring	
Butterbur mosaic (?)	Radish mosaic comovirus	carmovirus	Tomato black ring	
carlavirus	Raspberry ringspot	Pepper ringspot tobravirus	nepovirus	

Pepper ringspot tobravirus

nepovirus

	E 4-continued
Plant or Virus Name	Plant or Virus
Physalis mild chlorosis (?) luteovirus	Tomato bushy tombusvirus
Potato 14R (?) tobamovirus	Tomato golden
Potato black ringspot	bigeminivirus
nepovirus	Tomato mild m
Potato M carlavirus Potato mop-top furovirus	potyvirus Tomato mosaic
Potato T trichovirus	Tomato mottle
Potato U nepovirus	bigeminivirus
Radish mosaic comovirus	Tomato Peru p
Raspberry ringspot nepovirus	Tomato ringspo Tomato spotted
Red clover necrotic mosaic	tospovirus
dianthovirus	Tomato top neo
Ribgrass mosaic tobamovirus	nepovirus Tomato yellow
Rubus Chinese seed-borne	bigeminivirus
(?) nepovirus	Tomato yellow
Sowbane mosaic	bigeminivirus
sobemovirus Soybean dwarf luteovirus	Tulare apple m ilarvirus
Spinach latent ilarvirus	Tulip chlorotic
Strawberry latent ringspot	potyvirus
(?) nepovirus	Tulip halo neci
Subterranean clover red leaf luteovirus	Turnip mosaic Turnip rosette :
Sunn-hemp mosaic	Ullucus mild n
tobamovirus	tobamovirus
Sweet potato mild mottle	Ullucus mosaic
ipomovirus Tobacco etch potyvirus	Watermelon mo potyvirus
Tobacco mosaic	Wild potato mo
tobamovirus	potyvirus
Tobacco necrosis necrovirus	Wisteria vein n
Tobacco rattle tobravirus	potyvirus Patunia x hybr
Tobacco ringspot nepovirus Tobacco streak ilarvirus	Petunia x hybr Common name
Tobacco stunt varicosavirus	Common garde
Tobacco yellow dwarf	Garden petunia
monogeminivirus Tomato black ring	Susceptible to: Abelia latent ty
nepovirus	Alfalfa mosaic
Tulip halo necrosis (?) virus	Alstroemeria (
Tulip X potexvirus	Alstroemeria m
Turnip mosaic potyvirus Viola mottle potexvirus	potyvirus Amaranthus lea
Spinacia oleracea	potyvirus
Common names:	Amaranthus me
Spinach Susceptible to:	potyvirus
Alfalfa mosaic alfamovirus	Aquilegia (?) p Arabis mosaic
Amaranthus leaf mottle	Arracacha A ne
potyvirus	Arracacha B (?
Arabis mosaic nepovirus Asparagus 3 potexvirus	Artichoke later Artichoke vein
Barley stripe mosaic	nepovirus
hordeivirus	Artichoke yello
Bean yellow mosaic	nepovirus
potyvirus Beet curly top	Asparagus 2 ila Bean yellow m
hybrigeminivirus	potyvirus
Beet leaf curl (?)	Beet curly top
rhabdovirus	hybrigeminivin
Beet mild yellowing luteovirus	Beet western y luteovirus
Beet mosaic potyvirus	Bidens mottle
Beet necrotic yellow vein	Black raspberry
furovirus	virus
Beet pseudo-yellows (?) closterovirus	Brinjal mild m potyvirus
Beet soil-borne furovirus	Broad bean V
Beet western yellows	Broad bean wil
luteovirus	Butterbur mosa
Beet yellows closterovirus	carlavirus

Plant or Virus Name Tomato bushy stunt tombusvirus Tomato golden mosaic bigeminivirus Tomato mild mottle (?) potyvirus Tomato mosaic tobamovirus Tomato mottle bigeminivirus Tomato Peru potyvirus Tomato ringspot nepovirus Tomato spotted wilt tospovirus Tomato top necrosis (?) nepovirus Tomato yellow leaf curl bigeminivirus Tomato yellow mosaic bigeminivirus Tulare apple mosaic ilarvirus Tulip chlorotic blotch potyvirus Tulip halo necrosis (?) virus Turnip mosaic potyvirus Turnip rosette sobemovirus Ullucus mild mottle tobamovirus Ullucus mosaic potyvirus Watermelon mosaic 2 potyvirus Wild potato mosaic potyvirus Wisteria vein mosaic potyvirus . Petunia x hybrida Common names: Common garden petunia; Garden petunia Susceptible to: Abelia latent tymovirus Alfalfa mosaic alfamovirus Alstroemeria (?) ilarvirus Alstroemeria mosaic potyvirus Amaranthus leaf mottle potyvirus Amaranthus mosaic (?) potyvirus Aquilegia (?) potyvirus Arabis mosaic nepovirus Arracacha A nepovirus Arracacha B (?) nepovirus Artichoke latent potyvirus Artichoke vein banding (?) nepovirus Artichoke yellow ringspot nepovirus Asparagus 2 ilarvirus Bean yellow mosaic potyvirus Beet curly top hybrigeminivirus Beet western yellows luteovirus Bidens mottle potyvirus Black raspberry necrosis virus Brinjal mild mosaic (?) potyvirus Broad bean V (?) potyvirus Broad bean wilt fabavirus Butterbur mosaic (?) carlavirus

Black raspberry necrosis virus Broad bean wilt fabavirus Canavalia maritima mosaic (?) potyvirus Carnation mottle carmovirus Carnation ringspot dianthovirus Carnation vein mottle potyvirus Celery latent (?) potyvirus Cherry leaf roll nepovirus Clover yellow mosaic potexvirus Clover yellow vein potyvirus Cowpea mild mottle (?) Carlavirus Cowpea mosaic comovirus Croton yellow vein mosaic bigeminivirus Cumcumber leaf spot carmovirus Cucumber mosaic cucumovirus Cycas necrotic stunt nepovirus Cymbidium ringspot tombusvirus Dandelion yellow mosaic sequivirus Daphne Y potyvirus Dogwood mosaic (?) nepovirus Elderberry latent (?) carmovirus Elm mottle ilarvirus Epirus cherry ourmiavirus Foxtail mosaic potexvirus Galinsoga mosaic carmovirus Habenaria mosaic (?) potyvirus Heracleum latent trichovirus Lettuce infectious yellows (?) closterovirus Lettuce mosaic potyvirus Lettuce necrotic yellows cytorhabdovirus Lettuce speckles mottle umbravirus Lucerne Australian latent nepovirus Lucerne Australian symptomless (?) nepovirus Lucerne transient streak sobemovirus Lychnis ringspot hordeivirus Melon Ourmia ourmiavirus Melothria mottle (?) potyvirus Milk vetch dwarf nanavirus Mulberry latent carlavirus Nandina mosaic (?) potexvirus Nicotiana velutina mosaic (?) furovirus Oat blue dwarf marafivirus Okra mosaic tymovirus Parietaria mottle ilarvirus Parsnip leafcurl virus Parsnip mosaic potyvirus

TABLE 4-continued

Plant or Virus Name Cacao necrosis nepovirus Caper latent carlavirus Carnation mottle carmovirus Cassava green mottle nepovirus Cassava Indian mosaic bigeminivirus Cassava Ivorian bacilliform ourmiavirus Celery latent (?) potyvirus Cherry leaf roll nepovirus Chicory yellow mottle nepovirus Chrysanthemum B carlavirus Citrus ringspot virus Cowpea chlorotic mottle bromovirus Cowpea mosaic comovirus Cowpea severe mosaic comovirus Croton yellow vein mosaic bigeminivirus Cucumber leaf spot carmovirus Cymbidium ringspot tombusvirus Datura distortion mosaic potyvirus . Datura innoxia Hungarian mosaic (?) potyvirus Datura mosaic (?) potyvirus Dogwood mosaic (?) nepovirus Eggplant green mosaic potyvirus Eggplant mosaic tymovirus Eggplant mottled dwarf nucleorhabdovirus Elderberry latent (?) carmovirus Elm mottle ilarvirus Epirus cherry ourmiavirus Galinsoga mosaic carmovirus Grapevine chrome mosaic nepovirus Grapevine fanleaf nepovirus Groundnut eyespot potyvirus Guar top necrosis virus Henbane mosaic potyvirus Hibiscus latent ringspot nepovirus Hibiscus yellow mosaic (?) tobamovirus Hippeastrum mosaic potyvirus Honeysuckle latent carlavirus Humulus japonicus ilarvirus Kyuri green mottle mosaic tobamovirus Lamium mild mottle fabavirus Lettuce infectious yellows (?) closterovirus Lettuce necrotic yellows cvtorhabdovirus Lilac chlorotic leafspot capillovirus Lilac mottle carlavirus Lisianthus necrosis (?)

Plant or Virus Name

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	
Parsnip yellow fleck	necrovirus	arborea; Cleome pungens	Potato mop-top furovirus	
equivirus	Lucerne Australian	Common names:	Potato U nepovirus	
atchouli mosaic potyvirus	symptomless (?) nepovirus	Spider-flower	Potato yellow mosaic	
ea early browning	Lucerne transient streak	Susceptible to:	bigeminivirus	
obravirus	sobemovirus	Turnip yellow mosaic	Primula mosaic potyvirus	
ea streak carlavirus	Lychnis ringspot	tymovirus	Prune dwarf ilarvirus	
eanut chlorotic streak	hordeivirus	Gloriosa rothschildiana	Prunus necrotic ringspot	
aulimovirus	Marigold mottle potyvirus	Synonyms:	ilarvirus	
eanut clump furovirus	Melandrium yellow fleck	Gloriosa superba; Gloriosa	Raspberry ringspot	
eanut mottle potyvirus	bromovirus	abyssinica; Gloriosa homblei;	nepovirus	
eanut stunt cucumovirus	Melilotus mosaic (?)	Gloriosa hybrid; Gloriosa simplex;	Ribgrass mosaic	
elargonium flower break	potyvirus	Gloriosa speciosa; Gloriosa	tobamovirus	
armovirus	Melon Ourmia ourmiavirus	virescens	Rose (?) tobamovirus	
elagonium line pattern (?)	Narcissus mosaic potexvirus	Common names:	Rubus Chinese seed-borne	
armovirus	Neckar River tombusvirus	Flame lily; Glory lily;	(?) nepovirus	
epper Moroccan	Olive latent ringspot	Climbing lily; Creeping lily	Solanum nodiflorum mottle	
ombusvirus	nepovirus	Susceptible to:	sobemovirus	
epper ringspot tobravirus	Olive latent 2 (?)	Gloriosa fleck (?)	Sonchus cytorhabdovirus	
etunia asteroid mosaic	ourmiavirus	nucleorhabdovirus	Soybean crinkle leaf (?)	
ombusvirus	Paprika mild mottle	Tradescantia zebrina	bigeminivirus	
hysalis mild chlorosis (?)	tobamovirus	Synonyms:	Soybean mild mosaic virus	
iteovirus	Parietaria mottle ilarvirus	Tradescantia pendula;	Soybean mosaic potyvirus	
otato 14R (?) tobamovirus	Parsnip yellow fleck	Zebrina pendula	Spinach latent ilarvirus	
otato T trichovirus	sequivirus	Common names:	Sunflower ringspot (?)	
	Passionfruit Sri Lankan		ilarvirus	
otato U nepovirus		Wandering-jew		
adish mosaic comovirus	mottle (?) potyvirus	Susceptible to:	Sunn-hemp mosaic	
aspberry ringspot	Passionfruit woodiness	Tradescantia-Zebrina	tobamovirus	
eprovirus	potyvirus	potyvirus	Sweet potato mild mottle	
ed clover necrotic mosaic	Pea early browning	Chrysanthemum morifolium	ipomovirus	
ianthovirus	tobravirus	Synonyms:	Tamarillo mosaic potyvirus	
ibgrass mosaic	Pea seed-borne mosaic	Dendranthema x	Tobacco etch potyvirus	
ıbamovirus	potyvirus	grandiflorum; Anthemis	Tobacco leaf curl	
lose (?) tobamovirus	Peach enation (?) nepovirus	grandiflorum; Anthemis	bigeminivirus	
owbane mosaic	Peanut chlorotic streak	stipulacea; Chrysanthemum	Tobacco mild green mosaic	
obemovirus	caulimovirus	sinense; Chrysanthemum	tobamovirus	
oybean mild mosaic virus	Peanut clump furovirus	stipulaceum;	Tobacco rattle tobravirus	
pinach latent ilarvirus	Peanut green mosaic	Dendranthema x	Tobacco ringspot nepovirus	
pinach temperate	potyvirus	morifolium; Matricaria morifolia	Tobacco streak ilarvirus	
lphacryptovirus	Peanut stunt cucumovirus	Common names:	Tobacco stunt varicosavirus	
statice Y potyvirus	Peanut yellow spot	Florist's chrysanthemum;	Tobacco yellow vein (?)	
strawberry latent ringspot	tospovirus	Mum; Chrisanthemum	umbravirus	
?) nepovirus	Pelargonium line pattern (?)	Susceptible to:	Tomato black ring	
unflower ringspot (?)	carmovirus	Chrysanthemum B	nepovirus	
arvirus	Pelargonium vein clearing (?)	carlavirus	Tomato bushy stunt	
unn-hemp mosaic	cytorhabdovirus	Cucumber mosaic	tombusvirus	
bamovirus		cucumovirus		
	Pepper mild mottle		Tomato golden mosaic	
weet potato mild mottle	tobamovirus Bormor Moreocon	Oat blue dwarf marafivirus	bigeminivirus	
omovirus	Pepper Moroccan	Tomato aspermy	Tomato infectious chlorosis (?)	
obacco necrosis necrovirus	tombusvirus	cucumovirus	closterovirus	
obacco necrotic dwarf	Pepper ringspot tobravirus	Helianthus annuus	Tomato mosaic tobamovirus	
iteovirus	Pepper severe mosaic	Synonyms:	Tomato mottle	
obacco rattle tobravirus	potyvirus	Helianthus annuus var.	bigeminivirus	
obacco ringspot nepovirus	Pepper veinal mottle	macrocarpus; Helianthus	Tomato Peru potyvirus	
obacco streak ilarvirus	potyvirus	lenticularis	Tomato ringspot nepovirus	
obacco stunt varicosavirus	Petunia asteroid mosaic	Common names:	Tomato spotted wilt	
omato black ring	tombusvirus	Common annual sunflower;	tospovirus	
epovirus	Petunia vein clearing (?)	Sunflower; Hopi sunflower;	Tomato top necrosis (?)	
omato bushy stunt	caulimovirus	Common sunflower; Girasol	nepovirus	
mbusvirus	Physalis mosaic tymovirus	Susceptible to:	Tomato vein clearing	
omato spotted wilt	Pittosporum vein yellowing	Alfalfa mosaic alfamovirus	nucleorhabdovirus	
ospovirus	nucleorhabdovirus	Artichoke curly dwarf (?)	Tomato yellow mosaic	
ulip halo necrosis (?) virus	Plantago mottle tymovirus	potexvirus	bigeminivirus	
ulip X potexvirus	Plantain X potexvirus	Artichoke latent potyvirus	Tulip chlorotic blotch	
urnip mosaic potyvirus	Plum American line pattern	Beet western yellows	potyvirus	
	ilarvirus	luteovirus	Tulip halo necrosis (?) virus	
allota mosaic potyvirus				
iola mottle potexvirus	Plum pox potyvirus	Bidens mosaic potyvirus	Turnip mosaic potyvirus	
Vatermelon mosaic 2	Poplar mosaic carlavirus	Bidens mottle potyvirus	Ullucus mild mottle	
otyvirus	Potato 14R (?) tobamovirus	Cassia mild mosaic (?)	tobamovirus	
Vineberry latent virus	Potato Andean latent	carlavirus	Ullucus mosaic potyvirus	
Visteria vein mosaic	tymovirus	Cherry leaf roll nepovirus	White clover mosaic	
otyvirus	Potato aucuba mosaic	Citrus ringspot virus	potexvirus	
	potexvirus	Clover yellow mosaic	Wisteria vein mosaic	
leome spinosa	potexvirus			
Cleome spinosa Synonyms:	Potato black ringspot	potexvirus	potyvirus	

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TABLE 4-continued

TABLE 4-continued

TABLE 4-continued		TABLE	TABLE 4-continued		
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name		
ootyvirus	Synonyms:	Kalanchoe blossfeldiana	potyvirus		
Cucumber mosaic	Theobroma sativa	Synonyms:	Commelina X potexvirus		
cucumovirus	Common names:	Kalanchoe globulifera var.	Cowpea mild mottle (?)		
Cymbidium ringspot	Cacao; Chocolate-tree	coccinea	carlavirus		
ombusvirus	Susceptible to:	Susceptible to:	Cucumber mosaic		
Elm mottle ilarvirus	Cacao necrosis nepovirus	Kalanchoe latent carlavirus	cucumovirus		
Galinsoga mosaic	Cacao swollen shoot	Kalanchoe mosaic (?)	Cycas necrotic stunt		
carmovirus	badnavirus	potyvirus Kalanakas tau anattina	nepovirus		
Humulus japonicus ilarvirus	Cacao yellow mosaic	Kalanchoe top-spotting	Cymbidium ringspot		
Lettuce infectious yellows (?) closterovirus	tymovirus Cowpea mild mottle (?)	badnavirus Brassica napus var. napus	tombusvirus Dasheen mosaic potyvirus		
Maracuja mosaic (?)	carlavirus	Synonyms:	Dioscorea latent (?)		
zobamovirus	Okra mosaic tymovirus	Brassica campestris f.	potexvirus		
Melandrium yellow fleck	Tetragonia tetragonioides	annua; Brassica campestris f.	Dogwood mosaic (?)		
promovirus	Susceptible to:	biennis; Brassica napus f. annua;	nepovirus		
Patchouli mosaic potyvirus	Abelia latent tymovirus	Brassica napus f. biennis; Brassica			
eanut stunt cucumovirus	Alfalfa mosaic alfamovirus	napus ssp. oleifera;	nepovirus		
Pepper veinal mottle	Alstroemeria (?) ilarvirus	Brassica napus var. annua;	Foxtail mosaic potexvirus		
otyvirus	Alstroemeria mosaic	Brassica napus var. biennis;	Groundnut eyespot		
Physalis mosaic tymovirus	potyvirus	Brassica napus var. oleifera	potyvirus		
Prune dwarf ilarvirus	Alstroemeria streak (?)	Common names:	Habenaria mosaic (?)		
Prunus necrotic ringspot	potyvirus	Rape; Colza; Bird rape;	potyvirus		
larvirus	Amaranthus leaf mottle	Canola	Helenium S carlavirus		
Red clover necrotic mosaic	potyvirus	Susceptible to:	Heracleum latent trichovirus		
lianthovirus	Apple stem pitting virus	Watercress yellow spot	Hibiscus latent ringspot		
Sunflower crinkle (?)	Arabis mosaic nepovirus	virus	nepovirus		
ımbravirus	Arracacha A nepovirus	Brassica nigra	Hypochoeris mosaic (?)		
Sunflower mosaic (?)	Arracacha B (?) nepovirus	Synonyms:	furovirus		
ootyvirus	Arracacha latent (?)	Brassica nigra var.	Impatiens latent (?)		
Sunflower ringspot (?)	carlavirus	abyssinica; Sinapis nigra	potexvirus		
larvirus	Arracacha Y potyvirus	Common names:	Iris mild mosaic potyvirus		
Sunflower yellow blotch (?)	Asparagus 1 potyvirus	Black mustard	Kalanchoe isometric virus		
mbravirus	Asparagus 3 potexvirus	Susceptible to:	Kalanchoe latent carlavirus		
obacco necrosis necrovirus	Asystasia gangetica mottle	Beet western yellows	Lamium mild mottle		
obacco rattle tobravirus	(?) potyvirus	luteovirus	fabavirus		
obacco streak ilarvirus	Bean common mosaic	Ribgrass mosaic	Lettuce big-vein		
fomato black ring	potyvirus	tobamovirus	varicosavirus		
nepovirus	Bean yellow mosaic	Turnip mosaic potyvirus	Lettuce mosaic potyvirus		
Fomato spotted wilt	potyvirus Bast last such (2)	Turnip yellow mosaic	Lilac chlorotic leafspot		
ospovirus	Beet leaf curl (?)	tymovirus Citrullus un logaria	capillovirus		
Fropaeolum 2 potyvirus Convolvulus arvensis	rhabdovirus Root mild vollowing	Citrullus vulgaris Synonyms:	Lily X potexvirus		
Common names:	Beet mild yellowing luteovirus	<i>Citrullus lanatus</i> var.	Lisianthus necrosis (?) necrovirus		
Field bindweed	Beet mosaic potyvirus	lanatus; Citrullus aedulis; Citrullus			
nsusceptible to:	Beet necrotic yellow vein	lanatus var. caffer; Colocynthis	nepovirus		
Carnation vein mottle	furovirus	citrullus; Cucurbita citrullus	Lychnis ringspot		
otyvirus	Beet western yellows	Common names:	hordeivirus		
Cornus florida	luteovirus	Watermelon	Maclura mosaic		
Common names:	Beet yellows closterovirus	Susceptible to:	macluravirus		
Towering dogwood;	Broad bean necrosis	Cucumber green mottle	Malva veinal necrosis (?)		
American-boxwood	furovirus	mosaic tobamovirus	potexvirus		
Susceptible to:	Cacao necrosis nepovirus	Cucumber vein yellowing	Marigold mottle potyvirus		
Cherry leaf roll nepovirus	Cacao yellow mosaic	virus	Melandrium yellow fleck		
Dogwood mosaic (?)	tymovirus	Telfairia mosaic potyvirus	bromovirus		
epovirus	Carnation mottle	Watermelon chlorotic stunt	Melilotus mosaic (?)		
synonyms:	carmovirus	bigeminivirus	potyvirus		
Corylus avellana f. aurea;	Carnation ringspot	Wild cucumber mosaic	Melon Ourmia ourmiavirus		
Corylus avellana f. contorta;	dianthovirus	tymovirus	Narcissus latent		
Corylus avellana f. fusco-rubra;	Carnation vein mottle	Cucurbita maxima	macluravirus		
Corylus avellana f. heterophylla;	potyvirus	Common names:	Narcissus mosaic potexvirus		
Corylus avellana f.	Cassava green mottle	Squash; Pumpkin	Narcissus tip necrosis (?)		
pendula; Corylus avellana	nepovirus	Susceptible to:	carmovirus		
var. aurea; Corylus avellana var.	Cassava Ivorian bacilliform	Apple mosaic ilarvirus	Nerine potyvirus		
contorta; Corylus avellana var.	ourmiavirus	Bean yellow mosaic	Nerine X potexvirus		
	Cassia mild mosaic (?)	potyvirus	Odontoglossum ringspot		
heterophylla;	carlavirus	Beet curly top	tobamovirus		
Corylus avellana var.	Celery latent (?) potyvirus	hybrigeminivirus	Okra mosaic tymovirus		
pendula; Corylus heterophylla	Chickpea distortion mosaic	Cherry leaf roll nepovirus	Ornithogalum mosaic		
Common names:	potyvirus	Clover yellow mosaic	potyvirus		
European filbert; European	Chrysanthemum B	potexvirus	Parietaria mottle ilarvirus		
nazel; Avellana; Hazelnut	carlavirus	Cucumber leaf spot	Parsnip leafcurl virus		
Susceptible to:	Clover wound tumor	carmovirus	Parsnip yellow fleck		
Tulare apple mosaic ilarvirus	phytoreovirus Clover yellow vein	Cucumber mosaic cucumovirus	sequivirus Patchouli mottle (?)		

TABLE 4-continued

TABLE 4-continued

TABLE 4-continued		TABLE 4-continued	
Plant or Virus Name	Plant or Virus Name	Plant or Virus Name	Plant or Virus Name
Daphne X potexvirus	potyvirus	badnavirus	spontanea; Thea japonica
Elm mottle ilarvirus	Pea early browning	Yam mosaic potyvirus	Common names:
Eucharis mottle (?)	tobravirus	Vaccinium corymbosum	Common camellia
lepovirus	Pea mosaic potyvirus	Synonyms:	Susceptible to:
Frapevine fanleaf nepovirus	Pea seed-borne mosaic potyvirus	Vaccinium constablaei	Camellia yellow mottle (?)
Iumulus japonicus ilarvirus	Peach enation (?) nepovirus	Common names:	varicosavirus Thumbouria alata
Kyuri green mottle mosaic obamovirus	Peanut clump furovirus Peanut green mosaic	Highbush blueberry; Blueberry; American blueberry;	Thunbergia alata Common names:
ettuce infectious yellows	potyvirus	Swamp blueberry	Black-eyed-Susan-vine;
?) closterovirus	Peanut stunt cucumovirus	Susceptible to:	Ojitos-negros
isianthus necrosis (?)	Pelargonium flower break	Blueberry leaf mottle	Susceptible to:
ecrovirus	carmovirus	nepovirus	Datura yellow vein
faracuja mosaic (?)	Pelargonium line pattern (?)	Blueberry necrotic shock	nucleorhabdovirus
obamovirus	carmovirus	ilarvirus	Prune dwarf ilarvirus
felandrium yellow fleck	Pepino mosaic potexvirus	Blueberry red ringspot	Daphne cneorum
romovirus	Pepper ringspot tobravirus	caulimovirus	Common names:
felon leaf curl	Plantago mottle tymovirus	Blueberry scorch carlavirus	Rose daphne; Garland
igeminivirus	Poplar mosaic carlavirus	Blueberry shoestring	flower
felothria mottle (?)	Potato 14R (?) tobamovirus	sobemovirus	Susceptible to:
otyvirus	Potato black ringspot	Croton bonplandianus	Daphne S (?) carlavirus
apaya ringspot potyvirus	nepovirus	Synonyms:	Daphne X potexvirus
ea seed-borne mosaic	Potato mop-top furovirus	Croton sparsiflorus	Daphne Y potyvirus
otyvirus	Potato U nepovirus	Susceptible to:	Corchorus olitorius
eanut stunt cucumovirus	Primula mosaic potyvirus	Croton yellow vein mosaic	Common names:
oplar mosaic carlavirus	Red clover necrotic mosaic	bigeminivirus	Nalta jute; Tossa jute; Tussa
rune dwarf ilarvirus	dianthovirus	Euphorbia marginata	jute
runus necrotic ringspot	Ribgrass mosaic	Synonyms:	Susceptible to:
arvirus	tobamovirus	Euphorbia variegata	Okra mosaic tymovirus
adish mosaic comovirus	Solanum nodiflorum mottle	Common names:	Tropaeolum majus
owbane mosaic	sobemovirus	Snow-on-the-mountain	Common names:
obemovirus	Soybean dwarf luteovirus	Susceptible to:	Garden nasturtium; Indian-
quash leaf curl igeminivirus	Spinach latent ilarvirus Strawberry latent ringspot	Beet curly top hybrigeminivirus	cress; Mastuerzo Susceptible to:
quash mosaic comovirus	(?) nepovirus	Dulcamara mottle	Alfalfa mosaic alfamovirus
trawberry latent ringspot	Sweet clover necrotic	tymovirus	Apple mosaic ilarvirus
?) nepovirus	mosaic dianthovirus	Poinsettia mosaic (?)	Arabis mosaic nepovirus
Sunflower ringspot (?)	Sweet potato mild mottle	tymovirus	Beet curly top
arvirus	ipomovirus	Watermelon mosaic 2	hybrigeminivirus
obacco necrosis necrovirus	Sweet potato ringspot (?)	potyvirus	Beet western yellows
obacco ringspot nepovirus	nepovirus	Quercus velutina	luteovirus
obacco streak ilarvirus	Tamus latent (?) potexvirus	Common names:	Broad bean wilt fabavirus
omato bushy stunt	Telfairia mosaic potyvirus	Black oak	Cherry leaf roll nepovirus
ombusvirus	Tobacco etch potyvirus	Susceptible to:	Clover mild mosaic virus
Vatermelon curly mottle	Tobacco necrosis necrovirus	Oak ringspot virus	Cucumber mosaic
igeminivirus	Tobacco ringspot nepovirus	Eustoma russellianum	cucumovirus
Vatermelon mosaic 1	Tobacco stunt varicosavirus	Synonyms:	Cymbidium mosaic
otyvirus	Tomato black ring	Bilamista grandiflora;	potexvirus
Vatermelon mosaic 2	nepovirus	Eustoma grandiflorum;	Cymbidium ringspot
otyvirus	Tomato bushy stunt	Lisianthius russellianus	tombusvirus
Vild cucumber mosaic	tombusvirus	Common names:	Lamium mild mottle
movirus	Tomato vein clearing	Bluebells; Prairie-gentian	fabavirus
ucchini yellow fleck	nucleorhabdovirus	Susceptible to:	Lettuce infectious yellows
otyvirus	Tulip chlorotic blotch	Bean yellow mosaic	(?) closterovirus
ucchini yellow mosaic	potyvirus	potyvirus	Melandrium yellow fleck
otyvirus	Tulip halo necrosis (?) virus	Lisianthus necrosis (?)	bromovirus
'ycas revoluta	Tulip X potexvirus	necrovirus	Nasturtium mosaic (?)
common names:	Turnip crinkle carmovirus	Pelargonium peltatum	potyvirus
ago cycas; Sotesu-nut	Turnip mosaic potyvirus	Synonyms:	Okra mosaic tymovirus
usceptible to:	Ullucus C comovirus	Geranium peltatum	Pea early browning
ycas necrotic stunt	Ullucus mild mottle	Common names:	tobravirus Poplar mossio carlavirus
epovirus <i>Dioscorea alata</i>	tobamovirus Ullucus mosaic potyvirus	Ivy geranium; Hanging geranium	Poplar mosaic carlavirus Red clover necrotic mosaic
	Vallota mosaic potyvirus	Susceptible to:	dianthovirus
ynonyms: <i>ioscorea rubella</i>	Viola mottle potexvirus	Pelargonium flower break	Ribgrass mosaic
Common names:	Watermelon mosaic 2	carmovirus	tobamovirus
am; Greater yam; Water	potyvirus	Pelargonium line pattern (?)	Strawberry latent ringspot
am; Winged yam; White yam;	Wineberry latent virus	carmovirus	(?) nepovirus
am; winged yam; white yam;	Wisteria vein mosaic	Pelargonium vein clearing	Sunn-hemp mosaic
am; Name-de-Agna	potyvirus	(?) cytorhabdovirus	tobamovirus
Susceptible to:	Camellia japonica	Pelargonium x domesticum	Tobacco rattle tobravirus
Dioscorea alata potyvirus	Synonyms:	Insusceptible to:	Tobacco ringspot nepovirus
	Synonyms: Camellia japonica var.	Aster chlorotic stunt (?)	Tomato black ring
		ASICE CHIOTOLIC SUULL [1]	TOHIALO DIACK HILLS
Dioscorea trifida (?) otyvirus	hortensis; Camellia japonica var.	carlavirus	nepovirus

TABLE 4-continued

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TABLE 4-continued

Plant or Virus Name potyvirus tospovirus Chrysanthemum B carlavirus Saintpaulia ionantha potexvirus Common names: African violet; Usambara Synonyms: violet Anethum sowa; Susceptible to: Carnation ringspot Common names: dianthovirus Saintpaulia leaf necrosis (?) rhabdovirus Endro Ribes nigrum Susceptible to: Common names: Black currant; Cassis nepovirus Susceptible to: Strawberry latent ringspot (?) nepovirus Hypericum perforatum Common names: Common St. John's-wort; sequivirus Klamathweed; St. John's-wort; Goatweed Common names: Insusceptible to: Carnation ringspot Finocchio; Hinojo dianthovirus Susceptible to: Hyacinthus orientalis Common names nucleorhabdovirus Common hyacinth Insusceptible to: Susceptible to: Hyacinth mosaic potyvirus luteovirus Crocus vernus Susceptible to: Iris severe mosaic potyvirus sequivirus Freesia refracta Common names: Synonyms: Freesia leichtlinii; Gladiolus refractus heliotrope Susceptible to: Susceptible to: Freesia leaf necrosis varicosavirus potyvirus Verbena hybrida Freesia mosaic potyvirus Gladiolus Common names: Susceptible to: Artichoke Italian latent verbena nepovirus Susceptible to: Bean yellow mosaic Carnation ringspot potyvirus dianthovirus Cycas necrotic stunt nepovirus potyvirus Narcissus latent . Viola odorata macluravirus Common names: Iris Susceptible to: Garden violet Iris mild mosaic potyvirus Susceptible to: Iris severe mosaic potyvirus Tulip X potexvirus Juglans regia Synonyms: Vitis vinifera Juglans duclouxiana; Common names: Juglans fallax; Juglans kamaonica; European grape; Wine Juglans orientis; Juglans regia ssp. grape; Vid kamaonica; Juglans regia var. Susceptible to: orientis; Juglans regia var. sinensis; Juglans sinensis nepovirus Common names: English walnut; Persian walnut; Nogal susceptible to: Cherry leaf roll nepovirus tombusvirus Leguminosae Insusceptible to: nepovirus Voandzeia necrotic mosaic Grapevine chrome mosaic tymovirus

Plant or Virus Name Tropaeolum 2 potyvirus White clover mosaic -Anethum graveolens Peucedanum graveolens Dill; Dill seed; Garden dill; Eneldo; Aneto; Fenouil-batard; Artichoke yellow ringspot Carrot mottle umbravirus Carrot red leaf luteovirus Celery mosaic potyvirus Heracleum latent trichovirus Parsnip yellow fleck Foeniculum vulgare Fennel; Florence fennel; Coriander feathery red vein Celery yellow spot (?) Heracleum latent trichovirus Parsnip yellow fleck Valeriana officinalis Common valeriana; Garden-Watermelon mosaic 2 Garden verbena; Florist's Melilotus mosaic (?) English violet; Sweet violet; Viola mottle potexvirus Arabis mosaic nepovirus Artichoke Italian latent Grapevine A (?) trichovirus Grapevine ajinashika disease (?) luteovirus Grapevine Algerian latent Grapevine B (?) trichovirus Grapevine Bulgarian latent

TABLE 4-continued

Plant or Virus Name	Plant or Virus Name		
Mimosa pudica	nepovirus		
Common names:	Grapevine corky bark-		
Sensitive-plant; Touch-me-	associated (?) closterovirus		
not; Shame plant	Grapevine fanleaf nepovirus		
Insusceptible to:	Grapevine fleck virus		
Mimosa mosaic virus	Grapevine leafroll-		
Soybean mosaic potyvirus	associated (?) closteroviruses		
Lilium	Grapevine line pattern (?)		
Susceptible to:	ilarvirus		
Lily mottle potyvirus	Grapevine stem pitting		
Tomato aspermy	associated closterovirus		
cucumovirus	Grapevine stunt virus		
Tulip breaking potyvirus	Petunia asteroid mosaic		
Tulipa	tombusvirus		
Susceptible to:	Strawberry latent ringspot		
Arabis mosaic nepovirus	(?) nepovirus		
Tobacco rattle tobravirus	Zingiber officinale		
Tomato black ring	Synonyms:		
nepovirus	Amomum zingiber		
Tomato bushy stunt	Common names:		
tombusvirus	Ginger; Jengibre		
	Susceptible to:		
	Ginger chlorotic fleck (?)		
	sobemovirus		

[0126] Overview of Bioinformatics Methods

[0127] A. Phred, Phrap and Consed

[0128] Phred, Phrap and Consed are a set of programs which read DNA sequencer traces, make base calls, assemble the shotgun DNA sequence data and analyze the sequence regions that are likely to contribute to errors. Phred is the initial program used to read the sequencer trace data, call the bases and assign quality values to the bases. Phred uses a Fourier-based method to examine the base traces generated by the sequencer. The output files from Phred are written in FASTA, phd or scf format. Phrap is used to assemble contiguous sequences from only the highest quality portion of the sequence data output by Phred. Phrap is amenable to high-throughput data collection. Finally, Consed is used as a "finishing tool" to assign error probabilities to the sequence data. Detailed description of the Phred, Phrap and Consed software and its use can be found in the following references which are hereby incorporated herein by reference: Ewing, B., Hillier, L., Wendl, M. C. and Green, P. (1998) "Base-calling of automated sequencer traces using Phred. I. Accuracy assessment." Genome Res. 8: 175-178; Ewing, B. and Green, P. (1998) "Base-calling of automated sequencer traces using Phred. II. Error probabilities." Genome Res. 8:186-194; Gordon, D., Abajian, C. and Green, P. (1998) "Consed: a graphical tool for sequence finishing." Genome Res. 8: 195-202.

[0129] B. BLAST

[0130] The BLAST ("Basic Local Alignment Search Tool") set of programs may be used to compare the large numbers of sequences and obtain homologies to known protein families. These homologies provide information regarding the function of newly sequenced genes. Detailed description of the BLAST software and its uses can be found in the following references which are hereby incorporated herein by reference: Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990) "Basic Local Alignment Search Tool." J. Mol. Biol. 215: 403-410; Altschul, S. F. (1991) "Amino acid subsitution matrices from an informatics theoretic perspective." *J. Mol. Biol.* 219: 555-565.

[0131] Generally, BLAST performs sequence similarity searching and is divided into 5 basic programs: (1) BLASTP compares an amino acid sequence to a protein sequence database; (2) BLASTN compares a nucleotide sequence to a nucleic acid sequence database; (3) BLASTX compares translated protein sequences done in 6 frames to a protein sequence database; (4) TBLASTN compares a protein sequence to a nucleotide sequence database that is translated into all 6 reading frames; (5) TBLASTX compares the 6 frame translated protein sequence database. Programs (3)-(5) may be used to identify weak similarities in nucleic acid sequence.

[0132] The BLAST program is based on the High Segment Pair (HSP), two sequence fragments of arbitrary but equal length whose alignment is locally maximized and whose alignment meets or exceeds a cutoff threshold. BLAST determines multiple HSP sets statistically using "sum" statistics. The score of the HSP is then related to its expected chance of frequency of occurrence, E. The value, E, is dependent on several factors such as the scoring system, residue composition of sequences, length of query sequence and total length of database. In the output file will be listed these E values, these are typically in a histogram format, and are useful in determining levels of statistical significance at the user's predefined expectation threshold. Finally, the Smallest Sum Probability, P(N) is the probability of observing the shown matched sequences by chance alone and is typically in the range of 0-1.

[0133] BLAST measures sequence similarity using a matrix of similarity scores for all possible pairs of residues and these specify scores for aligning pairs of amino acids. The matrix of choice for a specific use depends on several factors: the length of the query sequence and whether or not a close or distant relationship between sequences is suspected. Several matrices are available including PAM40, PAM120, PAM250, BLOSUM 62 and BLOSUM 50. Altschul et al. (1990) found PAM120 to be the most broadly sensitive matrix (i.e. point accepted mutation matrix per 100 residues). However, in some cases the PAM120 matrix may not find short but strong or long but weak similarities between sequences. In these cases, pairs of PAM matrices may be used, such as PAM40 and PAM 250, and the results compared. Typically, PAM 40 is used for database searching with a query of 9-21 residues long, while PAM 250 is used for lengths of 47-123.

[0134] The BLOSUM (Blocks Substitution Matrix) series of matrices are constructed based on percent identity between two sequence segments of interest. Thus, the BLO-SUM62 matrix is based on a matrix of sequence segments in which the members are less than 62% identical. BLO-SUM62 shows very good performance for BLAST searching. However, other BLOSUM matrices, like the PAM matrices, may be useful in other applications. For example, BLOSUM45 is particularly strong in profile searching.

[0135] C. FASTA

[0136] The FASTA suite of programs permits the evaluation of DNA and protein similarity based on local sequence alignment. The FASTA search algorithm utilizes Smith/ Waterman- and Needleman/Wunsch-based optimization methods. These algorithms consider all of the alignment possibilities between the query sequence and the library in the highest-scoring sequence regions. The search algorithm proceeds in four basic steps:

- **[0137]** 1). The identities or pairs of identities between the two DNA or protein sequences are determined. The ktup parameter, as set by the user, is operative and determines how many consecutive sequence identities are required to indicate a match.
- **[0138]** 2). The regions identified in step 1 are rescored using a PAM or BLOSUM matrix. This allows conservative replacements and runs of identities shorter than that specified by ktup to contribute to the similarity score.
- **[0139]** 3). The region with the single best scoring initial region is used to characterize pairwise similarity and these scores are used to rank the library sequences.
- **[0140]** 4). The highest scoring library sequences are aligned using the Smith-Waterman algorithm. This final comparison takes into account the possible alignments of the query and library sequence in the highest scoring region.

[0141] Further detailed description of the FASTA software and its use can be found in the following reference which is hereby incorporated herein by reference: Pearson, W. R. and Lipman, D. J. (1988) "Improved tools for biological sequence comparison."*Proc.Natl.Acad. Sci.* 85: 2444-2448.

[0142] D. Pfam

[0143] Despite the large number of different protein sequences determined through genomics-based approaches, relatively few structural and functional domains are known. Pfam is a computational method that utilizes a collection of multiple alignments and profile hidden Markov models of protein domain families to classify existing and newly found protein sequences into structural families. Detailed description of the Pfam software and its uses can be found in the following references which are hereby incorporated herein by reference: Sonhammer, E. L. L., Eddy, S. R. and Durbin, R. (1997) "Pfam: a comprehensive database of protein domain families based on seed alignments." Proteins: Structure, Function and Genetics 28: 405-420; Sonhammer, E. L. L., Eddy, S. R. Bimey, E., Bateman, A. and Durbin, R. (1998) "Pfam: multiple sequence alignments and HMMprofiles of protein domains."Nucleic Acids Res. 26: 320-322; Bateman, A., Birney, E., Durbin, R., Eddy, S. R. Finn, R. D. and Sonhammer, E. L. L. (1999) Nucleic Acids Res. 27: 260-262.

[0144] Pfam 3.1, the latest version, includes 54% of proteins in SWISS_PROT and SP-TrEMBL-5 as a match to the database and includes expectation values for matches. Pfam consists of parts A and B. Pfam-A, contains a hidden Markov model and includes curated families. Pfam-B, uses the Domainer program to cluster sequence segments not included in Pfam-A. Domainer uses pairwise homology data from Blastp to construct aligned families.

[0145] Alternative protein family databases that may be used include PRINTS and BLOCKS, which both are based on a set of ungapped blocks of aligned residues. However,

these programs typically contain short conserved regions whereas Pfam represents a library of complete domains that facilitates automated annotation. Comparisons of Pfam profiles may also be performed using genomic and EST data with the programs, Genewise and ESTwise, respectively. Both of these programs allow for introns and frameshifting errors.

[0146] E. BLOCKS

[0147] The determination of sequence relationships between unknown sequences and those that have been categorized can be problematic because background noise increases with the number of sequences, especially at a low level of similarity detection. One recent approach to this problem has been tested that efficiently detects and confirms weak or distant relationships among protein sequences based on a database of blocks. The BLOCKS database provides multiple alignments of sequences and contains blocks or protein motifs found in known families of proteins.

[0148] Other programs such as PRINTS and Prodom also provide alignments, however, the BLOCKS database differs in the manner in which the database was constructed. Construction of the BLOCKS database proceeds as follows: one starts with a group of sequences that presumably have one or more motifs in common, such as those from the PROSITE database. The PROTOMAT program then uses a motif finding program to scan sequences for similarity looking for spaced triplets of amino acids. The located blocks are then entered into the MOTOMAT program for block assembly. Weights are computed for all sequences. Following construction of a BLOCKS database one can use BLIMPS to perform searches of the BLOCKS database. Detailed description of the construction and use of a BLOCKS database can be found in the following references which are hereby incorporated herein by reference: Henikoff, S. and Henikoff, J. G. (1994) "Protein family classification based on searching a database of blocks." Genomics 19: 97-10; Henikoff, J. G. and Henikoff, S. (1996) "The BLOCKS database and its applications." Meth. Enz. 266: 88-105.

[0149] F. PRINTS

[0150] The PRINTS database of protein family fingerprints can be used in addition to BLOCKS and PROSITE. These databases are considered to be secondary databases because they diagnose the relationship between sequences that yield function information. Presently, however, it is not recommended that these databases be used alone. Rather, it is strongly suggested that these pattern databases be used in conjunction with each other so that a direct comparison of results can be made to analyze their robustness.

[0151] Generally, these programs utilize pattern recognition to discover motifs within protein sequences. However, PRINTS goes one step further, it takes into account not simply single motifs but several motifs simultaneously that might characterize a family signature. Other programs, such as PROSITE, rely on pattern recognition but are limited by the fact that query sequences must match them exactly. Thus, sequences that vary slightly will be missed. In contrast, the PRINTS database fingerprinting approach is capable of identifying distant relatives due to its reliance on the fact that sequences do not have match the query exactly. Instead they are scored according to how well they fit each motif in the signature. Another advantage of PRINTS is that it allows the user to search both PRINTS and PROSITE simultaneously. A detailed description of the use of PRINTS can be found in the following references which are hereby incorporated herein by reference:Attwood, T. K., Beck, M. E., Bleasly, A. J., Degtyarenko, K., Michie, A. D. and Parry-Smith, D. J. (1997) *Nucleic Acids Res.* 25: 212-216.

[0152] Related, Variant, Altered and Extended Nucleic Acid Sequences

[0153] In one embodiment, the invention provides a polypeptide comprising the amino acid sequence encoded by a cDNA identified by a polynucleotide sequence chosen from the group consisting of SEQ ID NO: 1-122. The invention also encompasses variant polypeptides which retain the functional activity of causing a dwarf phenotype in a plant. A preferred variant is one having at least 80%, more preferably 90%, and most preferably 95% amino acid sequence.

[0154] It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding the same polypeptide, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence, and all such variations are to be considered as being specifically disclosed.

[0155] It may be advantageous to produce nucleotide sequences encoding polypeptide or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding a polypeptide and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

[0156] The invention also encompasses production of DNA sequences having the function of causing a dwarf phenotype in a plant, or portions thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into such a sequence or any portion thereof.

[0157] Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the polynucleotide sequences shown in SEQ ID NO: 1-122, under various conditions of stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex or probe, as taught in Wahl, G. M. and S. L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A. R. (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency. [0158] Altered nucleic acid sequences causing a dwarf phenotype in a plant which are encompassed by the invention include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that is functionally equivalent. The encoded polypeptide may also contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and consequently remains functionally equivalent. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the functional activity is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

[0159] Also included within the scope of the present invention are alleles of the genes encoded by cDNAs identified by the polynucleotide sequences SEQ ID NO: 1-122. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

[0160] Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE® (US Biochemical Corporation, Cleveland, Ohio), TAQ® polymerase (U.S. Biochemical Corporation, Cleveland, Ohio), thermostable T7 polymerase (Amersham Pharmacia Biotech, Chicago, Ill.), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE® amplification system (Life Technologies, Rockville, Md.). Preferably, the process is automated with machines such as the MICRO-LAB® 2200 (Hamilton Company, Reno, Nev.), PTC200 DNA Engine thermal cycler (MJ Research, Watertown, Mass.) and the ABI 377[™] DNA sequencer (Perkin Elmer).

[0161] The nucleic acid sequences of the invention may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

[0162] Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using OLIGO 4.06 primer analysis software (National Biosciences Inc., Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

[0163] Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

[0164] Another method which may be used to retrieve unknown sequences is that of Parker, J. D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER[™] DNA Walking Kits libraries (Clontech, Palo Alto, Calif.) to walk in genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

[0165] When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

[0166] Capillary electrophoresis systems which are commercially available (e.g. from PE Biosystems, Inc., Foster City, Calif.) may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. GENOTYPER® and SEQUENCE NAVIGATOR® from PE Biosystems, Foster City, Calif.) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

[0167] Vectors, Engineering, and Expression of Sequences

[0168] In another embodiment of the invention, cDNA sequences or fragments thereof which have the function of causing a dwarf phenotype in a plant, or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of polypeptides in appropriate host cells.

Due to the inherent degeneracy of the genetic code, other polynucleotide sequences which encode substantially the same or a functionally equivalent polypeptide also may be produced and these sequences may be used to clone and express the polypeptide of interest.

[0169] As will be understood by those of skill in the art, it may be advantageous to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

[0170] The polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter their polypeptide encoding sequences for a variety of reasons, including but not limited to, introducing alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

[0171] In another embodiment of the invention, natural, modified, or recombinant polynucleotide sequences having the function of causing a dwarf phenotype in a plant may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of the dwarf phenotype, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the wild-type coding sequence and the heterologous protein sequence, so that the wild-type polypeptide may be cleaved and purified away from the heterologous moiety.

[0172] In another embodiment, polynucleotide sequences having the function of causing a dwarf phenotype in a plant may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the polypeptide product may be produced using chemical methods to synthesize the amino acid sequence. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI $431A^{TM}$ peptide synthesizer (PE Corporation, Norwalk, Conn.).

[0173] The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (see, e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, N.Y.). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; or Creighton, supra). Additionally, the amino acid sequence, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide. **[0174]** In order to express a biologically active polypeptide, the encoding nucleotide sequences or their functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

[0175] Methods which are well known to those skilled in the art may be used to construct expression vectors containing nucleic acid sequences and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y, both of which are hereby incorporated by reference herein.

[0176] A variety of expression vector/host systems may be utilized to contain and express sequences having the function of causing a dwarf phenotype in a plant. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus, CaMV; tobacco mosaic virus, TMV; brome mosaic virus) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

[0177] The "control elements" or "regulatory sequences" are those non-translated regions of the vector-enhancers, promoters, 5' and 3' translated regions-which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT® phagemid (Stratagene, La Jolla, Calif.) or PSPORT1[™] plasmid (Life Technologies, Inc., Rockville, Md.) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

[0178] In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the resulting gene product. For example, when large quantities of gene product are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifinctional *E.coli* cloning and expression vectors such as BLUESCRIPT[®] phagemid (Stratagene, La Jolla, Calif.), in which a sequence may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -ga-

lactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEMXTM vectors (Promega Corporation, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

[0179] In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

[0180] In cases where plant expression vectors are used, the expression of sequences having the function of causing a dwarf phenotype in a plant may be driven by any of a number of promoters. In a preferred embodiment, plant vectors are created using a recombinant plant virus containing a recombinant plant viral nucleic acid, as described in PCT publication WO 96/40867 which is hereby incorporated herein by reference. Subsequently, the recombinant plant viral nucleic acid sequences may be transcribed or expressed in the infected tissues of the plant host and the product of the coding sequences may be recovered from the plant, as described in WO 99/36516, which is hereby incorporated herein by reference.

[0181] An important feature of this embodiment is the use of recombinant plant viral nucleic acids which contain one or more non-native subgenomic promoters capable of transcribing or expressing adjacent nucleic acid sequences in the plant host and which result in replication and local and/or systemic spread in a compatible plant host. The recombinant plant viral nucleic acids have substantial sequence homology to plant viral nucleotide sequences and may be derived from an RNA, DNA, cDNA or a chemically synthesized RNA or DNA. A partial listing of suitable viruses is described below.

[0182] The first step in producing recombinant plant viral nucleic acids according to this particular embodiment is to modify the nucleotide sequences of the plant viral nucleotide sequence by known conventional techniques such that one or more non-native subgenomic promoters are inserted into the plant viral nucleic acid without destroying the biological function of the plant viral nucleic acid. The native coat protein coding sequence may be deleted in some embodiments, placed under the control of a non-native subgenomic promoter in other embodiments, or retained in a further embodiment. If it is deleted or otherwise inactivated, a non-native coat protein gene is inserted under control of one of the non-native subgenomic promoters, or optionally under control of the native coat protein gene subgenomic promoter. The non-native coat protein is capable of encapsidating the recombinant plant viral nucleic acid to produce a recombinant plant virus. Thus, the recombinant plant viral nucleic acid contains a coat protein coding sequence, which may be native or a nonnative coat protein coding sequence, under control of one of the native or non-native subgenomic promoters. The coat protein is involved in the systemic infection of the plant host.

[0183] Some of the viruses which meet this requirement include viruses from the tobamovirus group such as Tobacco Mosaic virus (TMV), Ribgrass Mosaic Virus (RGM), Cowpea Mosaic virus (CMV), Alfalfa Mosaic virus (AMV), Cucumber Green Mottle Mosaic virus watermelon strain (CGMMV-W) and Oat Mosaic virus (OMV) and viruses from the brome mosaic virus group such as Brome Mosaic virus (BMV), broad bean mottle virus and cowpea chlorotic mottle virus. Additional suitable viruses include Rice Necrosis virus (TGMV), Cassava latent virus (CLV) and maize streak virus (MSV). However, the invention should not be construed as limited to using these particular viruses, but rather the method of the present invention is contemplated to include all plant viruses at a minimum.

[0184] Other embodiments of plant vectors used for the expression of sequences having the function of stunting a plant include, for example, viral promoters such as the 35S and 19S promoters of CaMVused alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196.

[0185] An insect system may be used to express the polypeptides of the invention. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in Trichoplusia larvae. The sequences encoding the gene product may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or Trichoplusia larvae in which the gene product may be expressed (Engelhard, E. K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

[0186] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleic acid sequences of the invention may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the relevant gene product in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

[0187] Specific initiation signals may also be used to achieve more efficient translation of the nucleic acid sequences of the invention. Such signals include the ATG initiation codon and adjacent sequences. In cases where a sequence, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

[0188] In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

[0189] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express a specific gene product may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

[0190] Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk⁻ or aprt⁻ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection; for example, dhfr, which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150: 1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) Methods Mol. Biol. 55:121-131).

[0191] Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if a nucleic acid sequence of the invention is inserted within a marker gene sequence, recombinant cells containing that specific sequence can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

[0192] Alternatively, host cells which contain a nucleic acid sequence of the invention and which express its gene product may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

[0193] The presence of polynucleotide sequences of the invention can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of polynucleotide sequence of interest. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences of interest to detect transformants containing the relevant DNA or RNA. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

[0194] A variety of protocols for detecting and measuring the expression of a cDNA, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the protein is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn.) and Maddox, D. E. et al. (1983; J. Exp. Med. 158:1211-1216).

[0195] A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to the polynucleotide sequences of the invention include oligonucleotide labeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits from Pharmacia & Upjohn (Kalamazoo, Mich.), Promega Corporation (Madison, Wis.) and U.S. Biochemical Corp. (Cleveland, Ohio). Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

[0196] Host cells transformed with a polynucleotide sequence of the invention may be cultured under conditions suitable for the expression and recovery of protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of its corresponding polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join polynucleotide sequences of the invention to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS[™] extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (available from Invitrogen, San Diego, Calif.) between the purification domain and polypeptide of interest may be used to facilitate purification. One such expression vector provides for expression of a fusion protein comprising a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. et al. (1992, Prot. Exp. Purif 3: 263-281,) while the enterokinase cleavage site provides a means for purifying polypeptide of interest from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. et al. (1993; DNA Cell Biol. 12:441-453).

[0197] In addition to recombinant production, a fragment of a polypeptide of the invention may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A peptide synthesizer (Perkin Elmer). Various peptide fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

[0198] In additional embodiments, the nucleotide and amino acid sequences of the present invention may be incorporated into any molecular biology techniques yet to be developed, provided these new techniques rely on properties of nucleotide and amino acid sequences that are currently

known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

[0199] The following examples further illustrate the present invention. These examples are intended merely to be illustrative of the present invention and are not to be construed as being limiting. The examples are intended specifically to illustrate the various methods used to identify and characterize the cDNAs of the present invention and the method by which they can be used to cause a dwarf phenotype in a plant.

EXAMPLES

[0200] I. Construction and Characterization of a Normalized Arabidopsis cDNA library in GENEWARE® Vectors

[0201] A. Plant Tissue Generation:

[0202] Arabidopsis thaliana ecotype Columbia (0) seeds were sown and grown on PEAT LITE MIX (Speedling Inc., Sun City, Fla.) supplemented with NUTRICOTE fertilizer (Plantco Inc., Ontario, Canada). Plants were grown under a 16-hour light/8-hour dark cycle in an environmental controlled growth chamber. The temperature was set at 22° C. for daytime and 18° C. for nighttime. The entire plant, root, leaves and all aerial parts were collected 4 weeks post sowing. Tissue was washed in deionized water and frozen in liquid nitrogen.

[0203] B. RNA Extraction:

[0204] High quality total RNA is isolated using a hot borate method. All solutions were made in DEPC-treated, double-deionized water and autoclaved. All glassware, mortars, pestles, spatulas, and glass rods were baked at 400° C. for four hours. All plasticware was DEPC-treated for at least three hours and then autoclaved.

[0205] Thirty-five milliliters of XT buffer (0.2 M Na borate decahydrate, 30 mM EGTA, 1% SDS (w/v), 1% deoxycholate, sodium) per 10 grams of tissue was dispensed into 50 milliliter Falcon tubes. PVP-40, 000 was added to a final concentration of 2% (w/v). NP-40 was added to a final concentration of 1% (w/v). Tubes were placed in an 80° C. water bath. The mortar and pestles were then pre-cooled in liquid nitrogen. Proteinase K (0.5 mg/ml XT buffer) was dispensed into 250 ml centrifuge bottles and the bottles were then placed on ice.

[0206] The tissue was added to the pre-chilled mortar and pestle and ground to a fine powder. Working as quickly as possible, the tissue was transferred to a glass beaker using a spatula chilled in liquid nitrogen. DTT (1.54 mg/ml XT buffer) was added to the XT buffer/PVP/NP-40 buffer and was immediately added to the ground tissue. The tissue was homogenized using a polytron at level 5 for one minute. The homogenate was decanted into the 250 ml centrifuge bottle containing the proteinase K. The homogenate was incubated at 42° C., 100 rpm for 1.5 hours. Eighty microliters of 2M KCl/ml of XT buffer was added to the homogenate and gently swirled until mixed. The samples were then incubated on ice for one hour. The samples were centrifuged at 12,000× G in a BECKAN® JA-14 rotor (Beckman Instruments, Inc., Fullerton, Calif.) for 20 minutes at 4° C. to remove debris. The supernatant was then filtered through a funnel lined with sterile miracloth into a sterile 250 ml

centrifuge bottle. Eight molar LiCl was added to a final concentration of 2M LiCl and the samples were incubated on ice overnight.

[0207] Precipitated RNA was pelleted by centrifugation at 12,000× G in a BECKMAN® JA-14 rotor for 20 minutes (Beckman Instruments, Inc., Fullerton, Calif.) and the supernatant was discarded. The RNA pellet was washed in 5 milliliters of cold 2M LiCl in 30 ml centrifuge tubes. Glass rods and gentle vortexing were used to break and disperse the RNA pellet. The pellets were centrifuged in a Beckman JA-20 rotor for 10 krpm at 4° C. for 10 minutes. The supernatant was decanted. This wash step was repeated 3 times until the supernatant was relatively colorless. The RNA pellet was resuspended in 5 milliliters of 10 Tris-Cl (pH 7.5). The insoluble material was pelleted in a JA-17 at 10 k rpm for 10 minutes at 4° C. The supernatant was transferred to another 30 ml centrifuge tube and 0.1× volume of 2M K-acetate (pH 5.5) was added. The samples were incubated on ice for 15 minutes and centrifuged in a BECK-MAN® JA-17 rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 10 k rpm, 4° C., for 10 minutes to remove polysaccharides and insoluble material. The supernatant was transferred to a sterile 30 ml centrifuge tube and RNA was precipitated by adding 2.5× volumes of 100% ethanol. The RNA was precipitated overnight at -20° C. The precipitated RNA was pelleted by centrifugation at 9 krpm, 4° C. for 30 minutes in a JA-17 rotor. The RNA pellet was washed with 5 milliliters of cold 70% ethanol and centrifuged in a JA-17 rotor at 9 k rpm, 4° C. for 10 minutes. The residual ethanol was removed using a BECKMAN® speed vac (Beckman Instruments, Inc., Fullerton, Calif.). The RNA pellet was resuspended in 3 milliliters of DEPC-ddH₂O+1 mM EDTA. The RNA was precipitated with 0.1× volumes of 3M Naacetate pH 6.0 and 2× volumes of cold 100% ethanol. The RNA was put at -80° C. for storage. A BECKMAN® spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) was used to measure absorbance (A) at A_{260} and A_{280} . The A_{260} was used to determine concentration (40 μ g RNA/ ml=1 A_{260} absorbance unit) and the A_{260}/A_{280} ratio was used to determine the initial quality of the RNA (1.8 to 2.0 is good).

[0208] The yield of total RNA from 60 g of tissue is ~15 mg. Then, mRNA was isolated from total RNA using oligo $(dT)_{25}$ DYNABEADS® (Dynal, Inc., Lake Success, N.Y.). Typically, 1% of total RNA population can be recovered as mRNA in *Arabidopsis thaliana* whole plant and from 5 μ g of poly A⁺ RNA, approximate 4.5 μ g of single strand cDNA and 6.7 μ g of double strand cDNA was synthesized.

[0209] C. cDNA Synthesis:

[0210] Poly A⁺ RNA was purified from total RNA using the oligo $(dT)_{25}$ DYNABEADS® kit (Dynal, Inc., Lake Success, N.Y.) according to manufacturer's instructions. Briefly, DYNABEADS® was resuspended by mixing on a roller and transfer 600 μ l to an RNase free tube. The beads were further equilibriated with 2× binding buffer (20 mM Tris-HCl, pH 7.5, 1M LiCl, 2 mM EDTA) twice and resuspended in 200 μ l of 2× binding buffer. Total RNA 1 mg (200 μ l) was heated at 70° C. for 5 minutes and incubated with the above oligo (dT)₂₅ DYNABEADS® for 10 min at RT. The supernatant containing unbound rRNA and tRNA was subsequently removed by magnetic stand and washed twice with 1× wash buffer (10 mM Tris-HCl, pH 7.5, 0.15M

LiCl, 1 mM EDTA). The mRNA was eluted from the DYNABEADS® in ddH_2O and used as the starting material for double strand cDNA synthesis.

[0211] Double strand cDNA was synthesized either with NotI-(dT)₂₅ primer or on oligo $(dT)_{25}$ DYNABEADS® based on the manufacturer's instruction (Gibco-BRL superscript system). Typically, 5 μ g of poly A⁺ RNA was annealed and reverse transcribed at 37° C. with SUPERSCRIPT II reverse transcriptase (Stratagene, La Jolla, Calif.). For the non-normalized cDNA library, double stranded cDNAs were ligated to a 500 to 1000-fold molar excess SaII adaptor, restriction enzyme NotI digested and size-selected by column fractionation. Those cDNAs were then cloned directionally into the XhoI-NotI sites of the TMV expression vector, 1057 N/P.

[0212] D. Normalization Procedure:

[0213] For the normalized cDNA preparation, the supernatant was removed from the DYNABEADS® and the cDNA containing beads were washed twice with $1 \times TE$ buffer. To carry out the normalization process, the second strand cDNA were eluted from the beads. $100 \,\mu$ l of TE buffer was added to the beads and heated at 95° C. for 5 min and the supernatant was then collected on magnetic stand. The above procedure was repeated once to ensure complete elution. The yield of second strand cDNA was quantitated using a UV spectrophotometer.

[0214] First strand cDNA beads is combined with second strand cDNA in 4× SSC, 5× Denhardt's and 0.5% SDS for multiple rounds of short hybridization. Since the second strand cDNA was synthesized using the first strand cDNA as the template, approximately the same amount of first and second strand cDNAs were present in the hybridization reaction. Nine μ g of second strand cDNA in 200 μ l of 1× TE buffer was added to the cDNA driver (first strand cDNA on beads) in a screw cap tube. The reaction was heated at 95° C. for 5 min, then 60 μ l of 20× SSC, 30 μ l of 50× Denhardt's (1% of Ficoll, 1% of polyvinylpyrrolidone and 1% of bovine serum albumin) and 15 μ l of 10% SDS were added and the reaction was brought to 65° C. for 8 hours.

[0215] The beads and supernatant were separated at 65° C. by magnet. The supernatant was transferred to a fresh tube and kept at 65° C. The beads were regenerated by adding 200 μ l of ddH₂O and heated at 95° C. for 5 min. We collected the beads for the next round of hybridization and kept the solution containing the bound second strand cDNA for further analysis. The partially normalized second strand cDNA solution was added back to the regenerated beads and a return to another round of hybridization of 8 hours. This procedure was repeated 4-5 times.

[0216] E. Slot Blot Analysis:

[0217] To follow the process of cDNA normalization a rapid slot blot procedure was developed. Following sequencing of 960 cDNAs, 46 cDNAs were selected to follow the representation of various classes of cDNAs through the normalization procedure. Based on their frequency of appearance in the sequence, these clones represent transcripts of different expression levels (high, moderate and low). Ten nanograms of each cDNA were deposited onto a HYBONDTM-N⁺ membrane (Amersham Pharmacia Biotech, Chicago, Ill.) along with control vector (pBS) and water controls. DNA was denatured, neutralized, and sub-

sequently crosslinked into the membrane using UV-STRATALINKER[™] 2400 (Stratagene, La Jolla, Calif.).

[0218] cDNAs from either the non-normalized or normalized pool were labelled with ³²P and hybridized on the slot blot membrane overnight at 65° C. in 1% bovine serum albumin, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.5 M sodium phosphate (pH 7.2), and 7% sodium dodecyl sulfate (SDS). Then, blots were washed once in 1× SSC/ 0.2% SDS for 20 min at room temperature followed by two washes in 0.2× SSC/0.2% SDS for 20 min. at 65° C. The resulting membranes were then developed using a PHOS-PHORIMAGERTM (Amersham Pharmacia Biotech, Chicago, Ill.) and quantitated using available software.

[0219] F. Conversion of Single-Stranded Normalized cDNAs to Double-Stranded Form:

[0220] Second strand normalized cDNA in hybridization solution was purified by QIAQUICK[™] column (QIAGEN GmbH, Hilden, Germany) and eluted in 88 μ l of ddH₂O (total 1.2 μ g of DNA is recovered). One μ l (3 μ g) of NotI-oligo dT primer was added and heated at 95° C. for 5 min followed by cool down to 37° C. The first strand cDNA was extended with T7 DNA polymerase (Amersham Pharmacia Biotech, Chicago, Ill.) in the presence of dNTP in 120 *µ*l reaction at 37° C. for 1 hour. T4 DNA polymerase (NEB) was then used to polish the ends following the extension reaction for 5 min at 16° C. The resulting double strand cDNA was ethanol precipitated and ligated with 500- to 1 000-fold molar excess of Sall adaptor followed by NotI digestion. The resulting cDNAs were size-fractionated using a Clontech spin column 400 and the first two fractions that contained the cDNAs were pooled and used for the subsequent cloning process.

[0221] G. Construction of cDNA Libraries in GENEWARE® Vectors:

[0222] (+) Sense cDNA clones were prepared as follows. The Tobacco Mosaic Virus expression vector, 1 056GTN-AT9 was linearized with NotI and XhoI and a 900 bp stuffer DNA was removed. The presence of the stuffer DNA in between those two sites is to ensure the complete digestion by restriction enzymes and thus achieve the high cloning efficiency. The digested vector was gel purified and then used to set up ligation reaction with normalized cDNA SalI-NotI fragments to generate (+) sense cDNA clones.

[0223] (-) Sense cDNA clones were prepared as follows. The Tobacco Mosaic Virus expression vector 1057 NP also linearized with NotI and XhoI and a stuffer DNA fragment was removed. The digested vector was gel purified and used to set up ligation reaction to generate (-) sense strand library.

[0224] Each ligation was transformed into chemically competent *E. coli* cells, DH5 α according to manufacturer's instruction (Life Technologies, Rockville, Md.). Preliminary analysis of cloning efficiency was measured by plating of a small portion of the transformation, while archiving the majority for future applications. Vector-only ligations gave $\sim 2 \times 10^4$ cfu/µg vector and ligations with cDNA insertions gave $\sim 5 \times 10^5$ cfu/µg.

[0225] H. Analysis of Normalized cDNA Populations:

[0226] With each successive round of kinetic re-association, the total cDNA population is depleted thereby confirming the removal of a population of the cDNA from the mixture at each step. To further understand the consequences of this depletion and measure the relative normalization in cDNA representation following various stages of the kinetic re-association method, slot blots of 46 genes of varying representations were hybridized with probes made from non-normalized and normalized cDNA preparations. The resulting blots were then analyzed for representation by PHOSPHORIMAGER® analysis. The hybridization pattern of non-normalized cDNA to the gene array reveals a quite asymmetric representation with some genes hybridizing with great intensity while others showing no hybridization at all. The variance among hybridization intensities for each spot within the filter was measured by standard deviation and found to be 649. In order to analyze the cDNA fraction depleted from the mixture, the first strand magnetic bead matrix was eluted, a radioactive probe was generated and hybridized to a replica of the slot blot described above. The resulting hybridization intensities indicated that primarily those cDNAs of higher copy number were bound and removed from the normalized cDNA population, confirming that the depletion phenomenon correlated with removal of primarily high copy number cDNAs. The cDNA population not bound to first strand magnetic beads after 5 serial passages was collected, radioactive probe was generated and hybridized to a replica slot blot of known gene set described above. The resulting hybridization pattern (i.e. the relative intensity of the slots on the blot) was in striking contrast to that of the non-normalized cDNA and to that of the bound cDNA fraction. Assuming that the majority of the hybridization signal to the slot blot for the non-normalized cDNA blot results from hybridization to high abundance genes, an initial comparison can be made between the number of bound counts on the normalized versus non-normalized slot blots. This comparison is possible since each probe added to the blots was derived from the same quantity of cDNA material and an equal number of probe counts were applied to the blots. The non-normalized blot contained 17,898 counts while the normalized blot contained only 1494 counts. This represents a 12-fold reduction in overall signal indicating a significant reduction in high gene copy number in the normalized cDNA population.

[0227] When the hybridization intensity of the non-normalized cDNA probe to each gene is plotted against the relative number of counts (following subtraction of the pBS vector control intensity from each sample), there is almost a 4-log difference in sequence representation in the cDNA population and an overall variance in standard deviation of 649-fold. In contrast, the hybridization of normalized cDNA probe to each gene revealed an average of only 32-fold difference. This represents both a reduction in high copy cDNAs and an increased representation in low copy cDNAs by >3 logs. The variance between the most highly represented cDNA and lowest represented cDNA within the normalized cDNA population was ~1.5 logs. The above values characterizing the degree of library normalization are equivalent to those achieved by Soares, et al. (1994).

[0228] I. Analysis of GENEWARE® Clones:

[0229] To ascertain the cloning efficiency of normalized cDNA into each vector and the average insert size, 96 random colonies were picked and grown by standard methods. DNA was isolated from bacteria using a BIOROBOT[™] 9600 (QIAGEN GmbH, Hilden, Germany). DNA was digested with Not I and BsiWI restriction endonucleases

(recognition sites flank the cDNA insertion). The digestions were separated on agarose gels and visualized by ethidium bromide staining. The digestions revealed a vector religation background of ~4%. Ligations giving >75% insertions were passed as to quality control and more colonies were picked. Approximently 600 independent clones were analyzed by restriction digestion as described above. Interestingly, a similar percentage of vector background was detected ~4% and the average insert size in the vector was ~1 kb, with many inserts with 2 kb or greater sized inserts. Following analysis of DNA by restriction mapping, DNA was subjected to sequencing and further analysis.

[0230] J. Sequence Analysis of the Normalized Arabidopisis Library in GENEWARE®:

[0231] Initial analysis of non-normalized Arabidopsis cDNA library required the sequencing of 1709 independent clones. Three 96-well plates of randomly picked normalized Arabidopsis library in GENEWARE®[(-) sense] were initially sequenced by primer TP6 to yield 262 5' sequences and passed sequence quality control. Initially, internal cluster analysis was performed to identify identical sequences in this sequence subset. Analysis using BLASTN algorithm showed that of the 262 sequences analyzed, 252 were unique and only 10 were found to cluster into five two-member clusters. We then identified the redundancy of the sequences against the larger public databases. For cluster analysis, we used a very low BLASTX score criteria $(e=10^{-6})$ and compared all sequences against the GENBANK® nr database (United States Department of Health and Human Services). In this manner, we could derive the most information concerning the redundancy, gene type found and open reading frame status of all clones simultaneously. The low BLASTX score was used to allow all possible protein homologues to be identified. The clustering analysis revealed that of the 262 sequences there were 252 single member sequence clusters and five two-gene clusters. This represents 96% singletons from this sample size. The genes appearing more than once in the library varied from two different chlorophyll a/b binding proteins, lipid transport proteins to ferrodoxin-thioredoxin reductases. This result compares quite favorably to the 4 redundant clones (of one gene type) identified by Soares, et al. (1994) from 187 randomly picked clones from one normalized library.

[0232] Further analysis of the sequences from the GENEWARE® normalized cDNA library revealed that of the 262 sequences subjected to BLASTX search of the GENBANK® nr database, 29% of the sequences failed to show significant homology to any characterized protein or open reading frame (ORF). Of the 252 singletons in the library, 179 showed single hit to an identified ORF, while 73 showed no hit. These results suggest that, in spite of the well characterized nature of the sequence database quality libraries can still contain a high proportion of new expressed sequences.

[0233] The excellent representation and extremely low redundancy observed in these initial plates of normalized Arabidopsis cDNAs in GENEWARE® prompted us to sequence additional clones. This was important because there is often a significant bias in small sample sizes with regard to representation. A total of 1,151 sequences passed sequence quality control. Internal cluster analysis showed that ~260 multi-sequence clusters were present, with the

highest representation at 6 members and the majority with only 2 members (~150). About 600 unique clusters were identified from the total of 856 clusters from the 1151 sequences. Therefore, from the 1151 sequences analyzed, 1,010 unique genes were identified, or a 87.7% gene discovery rate. In contrast, internal cluster analysis of the non-normalized Arabidopsis cDNA sequences revealed ~840 multi-gene clusters with the highest represented cluster containing 27 members. Cluster analysis of the 1709 nonnormalized Arabidopsis cDNAs revealed clusters of 27 members and many other highly populated clusters, a dramatic difference from the normalized cDNAs.

[0234] Further comparison of 1,151 randomly chosen nonnormalized sequences for redundancy with the results from the 1,151 normalized population clearly indicated the positive effects of normalization and the greater number of unique genes identified from this normalized population. Many genes that have representations of >12 in the nonnormalized library have been reduced to 1-4 members in the normalized population. One chlorophyll a/b binding protein gene exhibited a reduction from 15 members in the nonnormalized population to 1 in the normalized library, whereas a gene encoding a distinct chlorophyll a/b binding protein showed less reduction in the normalized gene population. This observation is consistent with the conclusion that certain genes do not undergo the same degree of normalization compared with other genes.

[0235] Additional sequences from the normalized Arabidopsis library were obtained by sequence analysis. BLASTN analysis of the 1,343 normalized sequences revealed that 858 were represented in the Arabidopsis EST database, while the remaining 485 sequences were apparently unique, with no obvious homologue in the database. Of those sequences showing BLASTN hits, 43.6% showed coverage of the first through tenth base in the longest EST in the database. Furthermore, 242 of the 858 (28%) showed 5' sequences that were at the first base of the longest EST or longer. These data show that the cDNAs cloned into GENEWARE® are of significant quality and represent, in many cases, the longest 5' sequences obtained to date. To further ascertain the proportion of cDNAs containing fulllength protein open reading frames, we employed the ORF finder program used to analyze the ABRC library for sense clones. This algorithm checks for ATG sequences in the first 70 bases of a sequence and then scans for sequences lacking an in-frame stop codon for at least 300 nt downstream in the same frame. To understand the number of quality ORFs in a library, we used the ABRC library as a benchmark. Analysis of 11,957 sequences within the ABRC library with the ORF finder program revealed 3,207 hits (26.8%) with putative open reading frames. From the 1,343 sequences of the normalized Arabidopsis cDNA library in GENEWARE®, 907 (67.5%) were hits using the ORF finder program. Coupling the number of cDNAs that represent near the 5' end of the known RNA sequence (43.6%) with the number of clones that contain putative intact ORFs (67.5%) testifies to the quality and integrity of the cDNAs in the GENEWARE® vector. These data clearly indicate a high proportion of full-length clones.

[0236] K. Quantity of Normalized Arabidopsis cDNAs Cloned into GENEWARE® Vectors:

[0237] As previously described, the normalized Arabidopsis cDNA population was cloned into GENEWARE® vec-

tors in both the positive (+) and negative (-) sense direction to allow for both overexpression and gene knockout analysis. The total number of clones in the 1057 PN vector in negative orientation was 20,160. These were arrayed into 210 96-well glycerol stock plates. Likewise, 20,160 clones from the ligation of normalized Arabidopsis cDNA in sense orientation into 1056 GTN vector have been arrayed in 210 96-well glycerol stock plates. These numbers clearly show that the GENEWARE® vectors can be used as primary cloning vectors and that very complex libraries can be obtained in two orientations from a single pool on nonamplified normalized cDNA.

[0238] II. Construction of Tissue-Specific *N. benthamiana* cDNA Libraries

[0239] A. mRNA Isolation:

[0240] Leaf, root, flower, meristem, and pathogen-challenged leaf cDNA libraries were constructed. Total RNA samples from 10-5 μ g of the above tissues were isolated by TRIZOL reagent (Life Technologies, Rockville, Md.). The typical yield of total RNA was 1 mg. PolyA+RNA was purified from total RNA by DYNABEADS® oligo (T)₂₅. Purified mRNA was quantified by UV absorbance at OD₂₆₀. The typical yield of mRNA was 2% of total RNA. The purity was also determined by the ratio of OD₂₆₀/OD₂₈₀. The integrity of the samples has OD values of 1.8-2.0.

[0241] B. cDNA Synthesis:

[0242] cDNA was synthesized from mRNA using the SUPERSCRIPT® plasmid system (Life Technologies, Rockville, Md.) with cloning sites of NotI at the 3' end and SalI at the 5' end. After fractionation through a gel column to eliminate adapter fragments and short sequences, cDNA was cloned into both GENEWARE® vector p1057 NP and phagemid vector PSPORTTM in the multiple cloning region between NotI and XhoI sites. Over 20,000 recombinants were obtained for all of the tissue-specific libraries.

[0243] C. Library Analysis:

[0244] The quality of the libraries was evaluated by checking the insert size and percentage from representative 24 clones. Overall, the average insert size was above 1 kb, and the recombinant percentage was >95%.

[0245] III. Construction of Normalized *N. benthamiana* cDNA Library in GENEWARE® Vectors

[0246] A. cDNA Synthesis.

[0247] A pooled RNA source from the tissues described above was used to construct a normalized cDNA library. Total RNA samples were pooled in equal amounts first, then polyA+RNA was isolated by DYNABEADS® oligo (dT)25. The first strand cDNA was synthesized by the Smart III system (Clontech, Palo Alto, Calif.). During the synthesis, adapter sequences with Sfi1a and Sfi1b sites were introduced by the polyA priming at the 3' end, and 5' end by the template switch mechanism (Clontech, Palo Alto, Calif.). Eight μ g first strand cDNA was synthesized from 24 μ g mRNA. The yield and size were confirmed by UV absorbance and agarose gel electrophoresis.

[0248] B. Construction of Genomic DNA Driver.

[0249] Genomic DNA driver was constructed by immobilizing biotinylated DNA fragments onto streptavidin-

coated magnetic beads. Fifty μg genomic DNA was digested by EcoR1 and BamH1 followed by fill-in reaction using biotin-21-dUTP. The biotinylated fragments were denatured by boiling and immobilized onto DYNABEADS® by the conjugation of streptavidin and biotin.

[0250] C. Normalization Procedure.

[0251] Six μ g of the first strand cDNA was hybridized to 1 μ g of genomic DNA driver in 100 μ l of hybridization buffer (6× SSC, 0.1% SDS, 1× Denhardt's buffer) for 48 hours at 65° C. with constant rotation. After hybridization, the cDNA bound on genomic DNA beads was washed 3 times by 20 μ l 1× SSC/0.1% SDS at 65° C. for 15 min and one time by 0.1× SSC at room temperature. The bounded cDNA on the beads was then eluted in 10 μ l of fresh-made 0.1N NaOH from the beads and purified by using a QIAGEN DNA purification column (QIAGEN GmbH, Hilden, Germany), which yielded 110 ng of normalized cDNA fragments. The normalized first strand cDNA was converted to double strand cDNA in 4 cycles of PCR with Smart primers annealed to the 3' and 5'end adapter sequences.

[0252] D. Evaluation of Normalization Efficiency.

[0253] Ninety-six non-redundant cDNA clones selected from a randomly sequenced pool of 500 clones of a previously constructed whole seedling library were used to construct a nylon array. One hundred ng of the normalized cDNA fragments vs. the non-normalized fragments were radioactively labeled by 32 P and hybridized to DNA array nylon filters. Hybridization images and intensity data were acquired by a PHOSPHORIMAGER® (Amersham Pharmacia Biotech, Chicago, III.). Since the 96 clones on the nylon arrays represent different abundance classes of genes, the variance of hybridization intensity among these genes on the filter were measured by standard deviation before and after normalization. These results indicated that by using this type of normalization approach, we could achieve a 1 000-fold reduction in variance among this set of genes.

[0254] E. Cloning of Normalized cDNA into GENEWARE® Vector.

[0255] The normalized cDNA fragments were digested by Sfi1 endonuclease, which recognizes 8-bp sites with variable sequences in the middle 4 nucleotides. After size fractionation, the cDNA was ligated into GENEWARE® vector p1057 NP in antisense orientation and transformed into DH5 α cells. Over 50,000 recombinants were obtained for this normalized library. The percentage of insert and size were evaluated by Sfi digestion of randomly picked 96 clones followed by electrophoresis on 1% of agarose gel. The average insert size was 1.5 kb, and the percentage of insert was 98% with vector only insertions of >2%.

[0256] F. Sequence Analysis of Normalized cDNA Library.

[0257] As of the date of this report, 2 plates of 96 randomly picked clones have been sequenced from the 5' end of cDNA inserts. One hundred ninety-two quality sequences were obtained after trimming of vector sequences and other standard quality checking and filtering procedure, and subjected to BLASTX search in DNA and protein databases. Over 40% of these sequences had no hit in the databases. Clustering analysis was conducted based on

accession numbers of BLASTX matches among the 112 sequences that had hits in the databases. Only three genes (tumor-related protein, citrin, and rubit) appeared twice. All other members in this group appeared only once. This was a strong indication that this library is well-normalized. Sequence analysis also revealed that 68% of these 192 sequences had putative open reading frames using the ORF finder program (as described above), indicating possible full-length cDNA.

[0258] IV. DNA Preparation

[0259] A. High Throughput Clone Preparation.

[0260] Arraying of the ABRC library into GENEWARE® vectors occurred as previously discussed to obtain ~5,000 antisense and ~3,000 sense clones with minimal redundancy. The ligations were between highly purified and quality controlled GENEWARE® cloning vector plasmids and the corresponding fragments from each individual pool of ABRC clones. Cloning efficiencies were in the range of $1 \times 10^{\circ}$ to 5×10^{5} per μg of plasmid. Colonies were picked using a Flexys Colony Picker (The Sanger Centre, England) and manual methods. Colonies were applied to deep-well cell growth blocks (DWBs) and grown from 18-26 hours at 37° C. at ~500 rpm in the presence of ampicillin concentrations of 500 µg/ml. From the almost 9,000 colonies picked by the Flexys, >97% of the cultures successfully grew. DNA was prepared using the QIAGEN BIOROBOT 9600 DNA robots and QIAGEN 96-well manifolds (manual preparation) at a rate of 2,000 DNA preparations per day. The final throughput, during campaign production, estimated for each system was ~20 plates of 96 samples per day, per production line-robotic or manual. Such throughput could be sustained to generate 20-40,000 samples in a matter of one to two weeks of effort. During one ten day period, one hundred four (140) 96-well plates of DNA were produced.

[0261] B. Quality Control Methods:

[0262] DNA samples were subjected to quality control (QC) analysis by at least one of two methods: 1) restriction endonuclease digestion and analysis by agarose gel electrophoresis (all plates) or 2) UV spectroscopy to determine DNA quantitation for all 96 samples of a plate (statistical sampling of each days output). For UV analysis, an aliquot of the DNA samples from each plate was taken and measured using a Molecular Dynamics UV spectrometer in 96-well format (Molecular Dynamics, Sunnyvale, Calif.). DNA concentrations of 0.05-0.2 μ l with OD 260/280 ratios of 1.7+0.2 are expected. For DNA sequencing purposes (a downstream method to be used to analyze all "hit" samples), DNA quantity of 0.04-0.2 μ g/ μ l is desired. In general, plates that contain >25% of samples not conforming to this metric are rejected and new DNA for the plate must be generated once again. For conformation of the presence of insertions and full-length GENEWARE® vector, agarose gel electrophoresis of restriction endonuclease fragments was used. Aliquots of sixteen samples from each 96-well DNA plate were targeted for restriction digestion using Nco I and BstE II restriction endonucleases. Samples were separated on 1%agarose gels. Generally, plates that showed >25% of samples that were not full length or did not contain insertions were rejected. From a total of 140 96-well DNA plates prepared, 112 passed QC and were made available for generation of infectious units.

[0263] V. High-Throughput DNA Sequencing and Sequence Analysis Protocols

[0264] A. Generation of Raw Sequence Data and Filtering Protocols:

[0265] High-throughput sequencing was carried out using the PCT200® and TETRAD® PCR machines (MJ Research, Watertown, Mass.) in 96-well plate format in combination with two ABI 377^{TM} automated DNA sequencers (PE Corporation, Norwalk, CT). The throughput at present is six 96-well plates per day.

[0266] The electropherogram generated from sequencer by ABI Sequencing Analysis (version 3.3) was used to generate sequence in the text format using "Phred," which also gives a confidence score for each base call that reflect the error probability and the quality for that base. Crossmatch was used to mask the vector sequence. The low quality portion of the sequence (i.e. phred score lower than 20) was removed. The vector and the polyA or polyT were also removed from the raw sequence. The high quality, processed sequences with the processing information were stored in the database. Sequences were used for further bioinformatic analysis.

[0267] B. Sequence Data Analysis and Bioinformatics:

[0268] Once the filtering and the vector sequence removal steps are completed, the resulting sequences are subjected to database search. First, low sensitivity methods such as BLASTN and BLASTX can be used. For those sequences that have no hit, more sensitive methods, such as Blimps and Pfam can be used. To speed up the analysis process, appropriate filters may be used. For example, for EST sequences from a given cDNA library sequenced from the 5' end, an ATG filter can be used to make sure that only full-length cDNA will be analyzed. The filtered sequence can be translated in one frame rather than six frames for Pfam analysis.

[0269] The results from the database search are stored in the relational database and can be used for further analysis. For example, all the BLAST results can be stored in a relational table that contains Query, Score, pValue, Hit, Length, Annotation, Frame, Identity, Homology, Query Length, Subject Length, Database Queried and Method used to analyze. Any result can be queried and analyzed by the fields mentioned. A database link between the analysis result database and the laboratory information management system (LIMS) has been created so that the analysis result can be related to the experimental data.

[0270] C. Metabolic Pathway Analysis:

[0271] Many metabolic pathway databases have been constructed that group proteins based on their roles in a metabolic pathway. The basic identifiers for these proteins are E.C. numbers; therefore, the position of a given enzyme in a metabolic pathway may be determined based on its E.C. number. The E.C. number of a protein can be obtained by its Genbank ID. This approach can be used to assign the corresponding E.C. number to the hits found for each cDNA sequence. By querying the metabolic pathway using the E.C. number of a hit, a potential link between this cDNA sequence and the metabolic pathway may be established. Each link can be used as a building block for a plant metabolic pathway. This potential link between cDNA sequence and metabolic pathway provides a starting point to analyze the gene's role in a metabolic pathway.

[0272] In addition, we have created an interactive, queriable relational prokaryotic and eukaryotic metabolic pathway database. This metabolic pathway database was created by accessing all public sequences that have associated E.C. numbers, running HMMs (hidden Markov models) and other proprietary LSBC algorithms against these sequences, and classifying these sequences into protein families based on conserved domains (Pfam database assignments). Pfam is a database of multiple alignments of protein domains or conserved protein regions. It is assumed that they represent some evolutionary conserved structure which has implications for the protein's function. Pfam is actually formed in two separate ways. Pfam-A are accurate human crafted multiple alignments whereas Pfam-B is an automatic clustering of the rest of SWISSPROT and TrEMBL derived from the Prodom (http://www.toulouse.inra.fr/prodom.html) database. Each protein family has the following data: 1). A seed alignment which is a hand edited multiple alignment representing the domain; 2). A Hidden Markov Model (HMM) derived from the seed alignment which can be used to find new members of the domain and also take a set of sequences to realign them to the model; 3). A full alignment which is a automatic alignment of all the examples of the domain using the HMM to find and then align the sequences; and 4). An annotation file which contains a brief description of the domain, some parameters for Pfam methods, and links to other databases.

[0273] We have run HMMs and other LSBC algorithms against the LSBC Sequence Database and classified these sequences into protein families based on conserved domains, and relate these sequences back to public sequences for E.C. mapping to metabolic pathways. We have run HMMs and other LSBC algorithms against all sequenced microbial genomes and classified these sequences into protein families based on conserved domains, and relate these sequences back to public sequences for E.C. mapping to metabolic pathways. We have run HMMs and other LSBC algorithms against all sequences into protein families based on conserved domains, and relate these sequences back to public sequences for E.C. mapping to metabolic pathways. We further related the Arabidopsis, *N. benthamiana*, and Oryza clones to specific sites on metabolic pathways.

[0274] D. Sequence Analysis of Library Created from GENEWARE® Vectors:

[0275] Five hundred sixty-eight (568) independent clones were sequenced from the virus expression library and the clones from this library were analyzed by vector, N filters and BLAST analysis. Of the 568 initial sequences submitted for analysis, 131 were eliminated by the N-filter indicating that ~15% of the sequence were undetermined Ns. The remaining 437 sequences were then subjected to analysis for duplication within each set of submitted plates. Fifty-five (55) sequences were removed due to this duplication filter. These sequences were BLASTN searched against 539 sequences from the AtwpLNLH library in Lambda Zap II. Thirty percent (30%) of the sequences (i.e., 132 sequences) found a match in both libraries. From the original set of GENEWARE® clones, 305 were found to be unique with respect to the Lambda Zap II library. These sequences were then BLASTX-searched against non-redundant GENBANK . From the 305 submitted sequences, 173 sequences found solid hits in protein coding sequence as determined by hit criteria and 132 were found to be unique. Further BLASTN analysis showed a range of sequence homology, but many represented hits to BAC or chromosomal sequences. A wide range of sequences were found including, ribosomal proteins, photosystem reaction center proteins, fumarase and other general metabolism proteins, transcription factors, kinase homologs, omega-6 fatty acid desaturase and various hypothetical proteins. These results strongly suggest that little or no bias is introduced during the construction of cDNA libraries in GENEWARE®.

[0276] VI. Preparation of Infectious Units

[0277] DNA plates that pass QC testing were then moved to the next stage of the cycle, the generation of infectious units. In vitro RNA transcriptions have been optimized to produce maximal amounts of RNA in smaller volumes to reduce costs and increase the lifetime of a DNA preparation. A transcription mixture containing a 6-to-1 RNA cap structure-to-rGTP ratio, Ambion mMessage Machine buffer and enzyme mix (Ambion, Inc., Austin, Tex.) is delivered to a 96-well plate by the TECAN liquid handling robot (TECAN, Research Triangle Park, N.C.). To this reaction mix, the Robbins Scientific HYDRA 96-sample pipeting robot (Robbins Scientific, Sunnyvale, Calif.) delivers 2 µl of DNA solution. This final transcription reaction is incubated at 37° C. for 1.5 hours. Following incubation, the TECAN robot delivers 95 µl of a 100 mM Na/K PO₄ buffer containing TMV coat protein (devoid of all infectious RNA) to the transcription plate and it is incubated overnight. This incubation generates encapsidated transcripts, which are very stable at room temperature or 4° C. and amplified with regard to number of infectious units per μg of RNA transcript. The generation of infectious materials is measured by inoculation of GFP-expressing virus to systemic host or Nicotiana tabacum NN lines, incubation at permissive temperatures and counting of developing local lesions on inoculated leaves. Before addition of the TMV coat protein mixture, 0.5 μ l from 8 wells of each transcription plate is removed and analyzed by agarose gel electrophoresis. The presence of an RNA band of ~1.6 to 3.5 kb is strong evidence for a successful transcription. If >25% contain only lower molecular weight RNA bands, or if the band is diffuse <500 bp of dsDNA marker, the transcription plate is considered to have failed and removed from the stream of plates prepared for inoculation. During a two week period, 112 plates were transcribed and 108 plates were passed for plant inoculation in growth rooms and in the field.

[0278] VII. Plant Inoculation with Encapsidated RNA Transcripts

[0279] In order to prepare for plant inoculation, 90 μ l of each encapsidated RNA transcript sample and 90 μ l of FES transcript inoculation buffer (0.1 M glycine, 0.06 M K₂HPO₄, 1% sodium pyrophosphate, 1% diatomaceous earth and 1% silicon carbide) were combined in the wells of a new 96-well plate. The 96 well plate was then placed on ice.

[0280] Nicotiana benthamiana plants 14 days post sowing were removed from the greenhouse and brought into the laboratory. Humidity domes were placed over the plants to retain moisture. The RNA transcript sample was mixed by pipetting the solution prior to application to ensure that the silicon carbide and the diatomaceous earth were resuspended. The entire sample, 180 μ l, was drawn up and pipetted in equal aliquots (approximately 30 μ l), onto the

first two true leaves of three separate *Nicotiana benthamiana* plants. The mixture was spread across the leaf surface using a TexwipeTM CleanfoamTM swab (The Texwipe Co, Upper Saddle River, N.J.). The wiping action caused by the swab together with the silicon carbide in the buffer sufficiently abrades the leaves so as to allow the encapsidated RNA transcript to enter the plant cell structure. Other methods used for inoculation have included pipeting of encapsidation-FES mixture onto leaves and rubbing by hand, cotton swab or nylon inoculation wand. Alternatively, nylon inoculation wands may be incubated in the transcript-FES mixture for ~30 min to soak up ~15 μ l and then rubbed directly onto the leaves.

[0281] Once an entire 32 plant flat was inoculated, the plants were misted with deionized water and the humidity domes were replaced over them. The inoculated plants were retained in the laboratory for 6 hours and then returned to the greenhouse. Once in the greenhouse, the humidity domes were removed and the plants were misted a second time with deionized water.

[0282] VIII. Inoculated Plant Growth

[0283] Plants inoculated with encapsidated virus were grown in a greenhouse. Day length was set to 16 hours and shade curtains (33% transmittance) were used to reduce solar intensity. Whenever ambient light fell below $250 \,\mu$ mol m²s⁻¹, a 50:50 mixture of metal halide and sodium halide lamps (Sylvania), delivering an irradiance of approximately 250 μ mol m²s⁻¹, were used to provide supplemental lighting. Evaporative cooling and steam heat were used to regulate temperature, with a daytime set point of 27° C. and a nighttime set point of 22° C. The plants were irrigated with Hogland's fertilizer mix as required. Drainage water was collected and treated with 0.5% sodium hypochlorite for 10 minutes before discharging into the municipal sewer.

[0284] To allow space for increased plant size, the inoculated *N. benthamiana* were repositioned at seven days post-inoculation (dpi) so that they occupied twice their original area. At 13 dpi, the plants were examined visually for symptoms of TMV infection and were assigned a numerical score to indicate the extent of viral infection (0=no infection, 1=possible infection, 2=limited/late infection, 3=typical infection, 4=severe infection). At the same time, the plants were assigned a fate for harvest (typically the highest quality plant in each triplicate was assigned to metabolic screens and the second highest quality plant was assigned to focused screens). In cases where plant symptoms deviated substantially from those of plants inoculated with control vectors, a description of plant phenotype was recorded (as described below). At 14 dpi infected plants were harvested.

[0285] IX. Infectivity Analysis

[0286] The method to measure the infectivity of the transcript encapsidations was to inoculate a set of 96-well plates from both positive and negative sense clones and look for systemic virus movement and phenotype development. Of the 8,352 plants inoculated with unique encapsidated transcriptions, 6,266 became systemically infected for an infection rate of 76%. Overall, the majority of plates generated showed very good infection rates. As shown in a graph of the number of systemically infectious constructs per each individual plate plotted against plate number. The majority of plates had systemic rates >70% with one at 100%. Approxi-

mately 25 plates had infection rates ranging between 40 and 70% while only 6% (>5 plates) showed infection rates <45%.

[0287] A population of constructs did not show systemic infection on Nicotiana benthamiana plants. Analysis using the LIMS revealed a substantial correlation between a subset of inoculators and the transcription plates showing poor infection rates. These results strongly suggest that inoculation technique is critical for good infectivity although other possible causes could include poor DNA or transcription quality, or simply inoculation error. In some cases the constructs may be restricted to inoculated leaves by way of adverse influence of the gene insertion on virus replication and movement. For example, one observed healthy inoculated Nicotiana benthamiana plant exhibited clear chlorotic spots on inoculated leaves, yet no systemic symptoms. Other plants, not scored as infected in our LIMS, were observed to have subliminal infections in source tissues. It was clear that the properties of the genetic insertion had differing effects on virus phenotypic symptoms. Eighty-two of those constructs exhibiting poor systemic infection were re-inoculated into Nicotiana tobacum NN plants to test for local lesions. The presence of local lesions indicated infectious viral vectors. From this data, a statistical calculation can be made to determine the percentage of non-systemic infective constructs that are locally infectious. Plants were scored 6 days post-inoculation for the presence of localized necrotic lesions resulting from infection and localized movement of virus vectors on the inoculated leaves of the plants. Of the 82 constructs analyzed, 50 showed local lesions indicating the presence of infectious viral vectors. Based on the infection rate observed in Nicotiana benthamiana and NN tobacco plants, we estimate that 1,181 (~61%) of the constructs not showing systemic infection on Nicotiana benthamiania plants were still infectious and amenable to biochemical analysis.

[0288] X. Phenotypic Evaluation

[0289] At 13 dpi a visual examination was made to identify plants whose phenotype deviates substantially from plants infected with a GENEWARE® control. The phenotypically different plants were divided into regions (for example: shoot apical region, infected phloem source leaves, stem) and descriptive terms were applied to each region to document the visual observation. Additionally, a confirmation was made as to whether or not the operator considered the plant to be a "hit" and a numerical score was applied to document the phytotoxic/herbicide effect of the RNA insert (1=possible effect, 2=mild, 3=moderate, 4=severe).

[0290] A matrix-style phenotypic database was created using the LIMS software. The LIMS software allows all descriptive terms to be used for any major part of the plant and the capacity of sub-parts to be described. Notable phenotypic events are captured by description of individual plant parts. The matrix is configured in a Web-based page that allows one to score infection and phenotyping using a graphic replicated of the physical arrangement of plants in the growth room. This approach is rapid, allowing 96 plants to be described in detail as being infected, not infected with a detailed phenotype in ~15 min. Editing of output files can occur rapidly in MS Excel if desired. The output file is then loaded as CSV files into the LIMS where it is immediately available to Boolean query as to phenotype descriptors with

"and, or, not" statements. Images of infected plants are linked to the SeqIDs in the database so that the plant tray bar code (for infection), well position, SeqID, phenotype and picture all link together when a query is made. This is linked back to the sequence database for sequence annotation data. Using this system, 8,352 phenotypic observations were made in the period of two days and entered into the LIMS. Hundreds of interesting visual phenotypes were observed.

[0291] XI. Field-Scale Genomics

[0292] The effects of gene overexpression and gene silencing in plants may have dramatic differences when grown under different conditions. The Kentucky field test plots available to Biosource provides an opportunity to subject plants to substantially different growth conditions and thereby broaden the chances of detecting various types of "hits" in a genomics screen. To compare the ability of virus vectors to be applied under field conditions and under controlled growth room conditions, we inoculated, in duplicate, 960 positive-sense constructs on Nicotiana benthamiana plants grown in the field test plot in Owensboro, Ky. This activity was concurrent with inoculations and screens performed in Vacaville, Calif. Complete encapsidated transcription reactions were prepared at Large Scale Biology Corporation in Vacaville, Calif. and following incubation with TMV coat protein, FES buffer was added to each well. All samples in column 12 of each plate contained encapsidated transcripts of 1057 vector containing the GFP gene. The mixture was then overnight-mailed to Owensboro, Ky. where it was inoculated onto 4-5 week post-sowing plants by rubbing cotton swabs, pre-wetted by incubation with encapsidated transcript-FES mixture, on plant leaves. Plants were inoculated in duplicate. Plants were allowed to remain in the field for 4 weeks post-inoculation and then subjected to phenotypic analysis. Photographic documentation of the plants both pre- and post-inoculation was prepared. Plants were scored by visual evaluation as to number of infected plants compared with total number of plants inoculated. Of the 1920 plants inoculated, 1,712 (88%) showed systemic infections. More than 100 new phenotypes were noted in the field. Each was compared with the phenotype of the same construct inoculated into plants in Vacaville, Calif. growth rooms. Two new phenotypes are particularly noteworthy: two independent plants showed survival phenotypes under anaerobic conditions, whereas all neighbors had succumbed to root rot in a low spot in the field.

[0293] In order to evaluate the effect of gene silencing in Nicotiana tabacum plants, mRNA from Arabidopsis thaliana whole plants was subjected to fragment normalization such that small cDNA fragments were produced. The cDNA population showed high degree of normalization by hybridizations with known genes of variable expression and by comparison with non-normalized cDNA fragments. The average size of the normalized fragments in the GENEWARE® vectors was between 400-500 bp allowing facile movement of the recombinant viruses systemically in field Nicotiana tabacum c.v. MD609 plants. A total of 11 plates of DNA constructs (1056) were prepared, transcribed and encapsidated with GFP constructs integrated at every 12th position. These were mixed with FES and overnightmailed to Owensboro, Ky. These 1056 constructs were inoculated in duplicate (2112 total) on MD609 tobacco plants 11 weeks post-sowing. One set of the replicates (1056 plants) were scored by visual evaluation as to number of infected plants compared with total number of plants inoculated. Of the 1056 plants inoculated, 808 showed systemic infections, or 76.5% infection rate. "Hits" were determined by unusual visual symptoms and corresponding constructs will be characterized by DNA sequencing.

[0294] An uncharacterized GENEWARE® library comprised of 20,000 Arabidopsis thaliana normalized fragment cDNAs and 10,000 of Nicotiana benthamiana genomic DNA fragments was prepared and sprayed as a population on Nicotiana tabacum c.v. MD609 plants. The Arabidopsis cDNA library, ~10,000, was constructed by ligation into prepared GENEWARE® vectors and purified from pooled bacterial transformants and followed by pooled transcription. The remaining 10,000 cDNA fragments were individual clones prepared and transcribed independently and then mixed in a pooled encapsidation. The Nicotiana library was a prototype cell-free cloning library from restriction endonuclease fragmented gDNA of <500 bp in size. The number of clones corresponds to an approximation of the amount of DNA undergoing complete ligation. Transcriptions from each non-encapsidated library were inoculated separately into Nicotiana tabacum protoplasts and allowed to incubate for three days. Cells were lysed and libraries combined. The pool of cell lysates and encapsidated transcriptions containing viral libraries were shipped to Owensboro, KY where they were inoculated onto Nicotiana tabacum c.v. MD609 plants at 1, 1/10, 1/100 and 1/000 dilution of the mixed virion preparation (using 60 ml, 6 mls, 0.6 mls and 0.06 mls of the library respectively). Eight hundred (800) plants were spray-inoculated with each library virion dilution. Plants were visually scored and of the 3,200 plants inoculated, 1,304 showed visual symptoms 3 weeks postinfection. The infectivity rate varied from ~60% for the most concentrated inoculum to ~20% for the most dilute as would be expected due to dilution. Analysis will continue to define "Hits" by unusual visual symptoms and PCR amplification and DNA sequencing will characterize corresponding construct.

[0295] XII. GC/MS Metabolite Analysis

[0296] A. Harvest and Preparation of Tissues for Metabolic Screening

[0297] Fourteen dpi infected plants to be harvested were moved from the greenhouse to the laboratory. Plants were scanned and identified by a bar-code that linked the infected plant to the tissue sample. The infected tissue was cut off of the plant and placed in a corresponding centrifuge tube. A tungsten carbide ball was placed on top of the infected tissue sample. The tungsten carbide ball facilitates pulverization of plant tissue. The tubes and sample were stored on dry ice during the harvesting procedure. The samples were then stored at -70° C. Before conducting a metabolic screen, the tissue samples must be pulverized. The sample tubes were loaded into a KLECO pulverizer and pulverized to create a fine powder of the tissue sample. The tissue sample powder was then weighed out into a metabolic extraction vial.

[0298] B. FAME Analysis Procedure for FAME Screen.

[0299] Nicotiana benthamiana plants expressing genes of interest in RNA vectors were grown for 14 dpi as described above. Three leaf disks (0.5 cm in diameter) were placed in cell wells of a borosilicate 96-deepwell plate (Zinsser). 500 μ l of heptane was added to each well using a Biomek 2000

Laboratory Automation Workstation. The heptane/tissue samples were stirred on a Bodine magnetic stirrer. After 30 minutes, 50 μ l of 0.5N sodium methoxide in methanol was added to each well using the Biomek 2000. After 30 minutes of stirring, $10 \,\mu$ l of water was added to each well. Injections were made directly from the 96-deepwell plate into a Hewlett Packard gas chromatograph (GC) using a LEAP auto injector. The GC method involved a 2 μ l injection into a split/splitless injection port using a DB 23 narrow bore column (15 M, 0.25 I.D.). The oven temperature was isothermic at 170° C. The injector temperature was 230° C. and the detector (flame ionization) temperature was 240° C. The run time was 5 minutes, with an equilibration time of 0.5 minutes. The split ratio was 20:1 and the helium flow rate was held at a constant pressure of 19 psi. This GC method allowed for separation and quantification of fatty acid methyl esters which included C16:0,C16:1,C18:0,C18:1, C18:2, and C18:3. Using a dual column GC, four 96-well plates could be sampled in less than 24 hours.

[0300] The following sequences exhibited a positive FAME result (had altered levels of the fatty acids assayed): SEQ ID NOS: 7, 53, and 92. The result of the FAME analysis for SEQ ID NO:92 is shown in Table 5. Table 5 shows the relative percent amounts of fatty acids found in plants transfected with a viral vector comprising SEQ ID NO: 92. An increase in 16:0 fatty acids was observed in 3 of the 5 samples assayed. Table 6 shows the relative percent amounts of fatty acids found in plants transfected with SEQ ID NO: 7 and 53.

TABLE 5

FAME Profile										
Sample	16:0	16:1	unk	16:3	unk	18:0	18:1	18:2	18:3	unk
1	24.7	3.4	1.1	3.2	2.6	2.6	3.3	9.2	47.8	2.0
2	20.1	2.9	0.8	4.6	2.9	3.5	7.1	9.2	46.7	2.3
3	17.6	1.8	1.0	3.5	2.9	2.2	6.0	11.8	50.4	2.7
4	23.3	1.9	1.0	3.1	4.6	3.8	8.9	10.6	37.6	5.3
5	23.0	2.6	0.7	3.5	1.6	2.3	3.8	8.1	52.9	1.6
control	19.6	2.8	1.1	3.3	1.8	1.8	3.1	12.0	53.6	1.0
control	18.4	2.7	1.1	3.3	1.7	1.7	3.1	11.3	55.4	1.3

[0301]

TABLE 6

FAME Profile										
Sample	16:0	16:1	unk	16:3	unk	18:0	18:1	18:2	18:3	unk
SEQ ID NO: 53	23.0	3.5	1.9	2.6	1.7	2	3.3	11.7	49.1	1.3
SEQ ID NO: 7	25.7	3.4	1.3	1.8	0.8	2.3	2.1	8	54.7	0
control	18.7	2.8	1.2	3.8	1.4	1.5	4.2	10.7	55	0.6

[0302] C. Insect Control Bioassays.

[0303] Nicotiana benthamiana plants expressing genes of interest in RNA viral vectors were grown for 14 dpi as described previously. Fresh leaf tissue (sample size ~2.5 cm diameter) was excised from the base of infected leaves using a scalpel and placed in insect-rearing tray (Bio RT32, C-D

International) wells containing 3 ml of 2% agar. Using a small paintbrush to handle insects, 2 first-instar larvae of tobacco hornworm (Manduca sexta) were placed in each well and trays were sealed using vented covers. Trays were then incubated at 28 C with 48% humidity for 72 hours with a 12-hour photoperiod. Following incubation, samples were scored for mortality and leaf damage according to the following criteria: mortality, 0=0 dead/2 alive; 1=1 dead/1 alive; 2=2 dead/0 alive; leaf damage, 0=0 to 20% leaf consumed; 1=21 to 40% leaf consumed; 2=41 to 60% leaf consumed; 3=61 to 80% leaf consumed; and 4=81 to 100% leaf consumed. Following scoring, insects were weighed on an analytical balance and photographed using a digital camera.

[0304] The following sequences exhibited a positive insect control phenotype: SEQ ID NOs: 3, 5, 7, 27, 32, 37, 59, 80, 92, 103, 106, 108, 109, 110, and 111.

[0305] D. Carbohydrate Screen.

[0306] The dry residue was transferred from the extracting cartridge (10-20 mg) into a 100×13 mm glass tube containing 0.5 ml of 0.5 N HCI in methanol and 0.12 ml of methyl acetate and then sealed (Teflon coated screw cap) under nitrogen and heated for 16 hours at 80° C. The liquid phase was then transferred using an 8-channel pipetter (Matrix) to a glass insert supported by a 96 well aluminum block plate (Modem Metal Craft) and evaporated to dryness (Concentrator Evaparray). The methyl-glycosides and methyl-gly-

coside methyl esters were silylated in 0.1 ml pyridine and 0.1 ml BSTFA+1% TMCS at room temperature for one hour. The sample generated was analyzed on a DB 1 capillary column (15 meters) with an 11 minute program temperature (from 160° C. to 190° C. at 5° C./min and 190° C. to 298° C. at 36° C./minute and hold 2 minutes) and 3 minutes equilibration time. The following components of the plant

cell wall were identified in the tobacco sample: arabinose, rhamnose, xylose, galactose, galacturonic acid, mannose, glucuronic acid and glucose.

[0307] E. GC/MS Metabolite Analysis:

[0308] A 3 mm tungsten carbide ball bearing was placed into each well of a 96-well deep well block and 300 μ l of grinding buffer (2 mM NaOH, 1 mM PMSF, 10 mM beta-mercaptoethanol, and deuterium-labeled compounds) was added to each well. A 13 mm circle (~20 mg) leaf disc plug from ~4 week old Nicotiana benthamiana (2 week post-inoculation) apical leaves were placed into the 96-well microtiter deepwell plate. The plate was tightly sealed and placed on a mechanical shaker (paint mixer, up to four at a time) for 2 min, then rotated 180° and shaken for an additional 2 min. Subsequently, the samples were spun for 10 min at 3200 RPM in a refrigerated (15° C.) centrifuge equipped for microtiter plates. Following centrifugation, the 96-well plate containing the homogenized samples was placed on a TECAN GENESIS RSP 200 (TECAN, Research Triangle Park, N.C.) liquid handler/robotics system. Both Logic and Gemini software were used to control the TECAN liquid handler. Approximately 200 μ l was transferred to a pre-conditioned (1 ml MeOH followed by 1 ml of distilled deionized H₂O) Waters 96-well Oasis HLB solid phase extraction (SPE) plate by the TECAN liquid handler for metabolite analysis by GC/MS. The Waters Extraction Plate Manifold Kit and a vacuum not greater than 5 mm Hg was used to aspirate plant samples from SPE plate into a waste reservoir. The SPE plate was then washed with 1 ml of 5% MeOH in H₂O by aspirating into waste reservoir and compounds eluted from SP resin with 350 μ l of MeOH into a 96-well collection plate. Samples were then transferred to GC autosampler vials, capped and stored in the freezer at 80° C. for metabolite analysis.

[0309] An internal standard solution was prepared by making a stock solution at a concentration of 1 μ l (using compound density). Grinding buffer (2 mM NaOH above) with the internal standard was prepared at a concentration of 10 ng/ μ l for each (3,000 ng/300 μ l) to yield a concentration equivalent of approximately 150 ng/mg wet weight of plant tissue. Following extraction of plant material, this solution was transferred to the SPE plate by the TECAN liquid handler and extracted with 350 μ l of MeOH. Approximately 20 μ l of the sample will be injected onto a 30 m×0.32 mm DB-WAX (1 μ m film thickness) GC column with a large volume injector during the preliminary study. The GC column oven was temperature held at 35 C for 5 min, then programmed at 2.5° C./min to 250° C. and held for 15 min.

[0310] Samples that contained peaks that were present in altered levels relative to control samples as identified from chromatograms were further analysis using mass spectroscopy. Samples that were transfected with the following nucleic acid sequences were found to have altered metabolic profiles: SEQ ID NO: 43, 50, 81, 85, and 92. Table 7 shows the retention time and % change in peaks relative to controls for several sequences. Table 7 also shows the identity of the peaks as determined by mass spectroscopy.

TABLE 7

Metabolic Profiles				
SEQ ID NO	RT (MIN)	% Change	Compound	
43	10.68	+130	Malic Acid	
43	11.63	+250	Ribonic Acid; Gamma- lactone	
43	12.93	+260	Quinic Acid	
43	14.12	+120	Inositol	
81	10.67	+300	Malic Acid	
81	10.87	+150	L-Aspartic Acid	
81	10.92	+80	5-Oxo-L-Proline	
			(pyroglutamic)	
81	12.48	+100	Ribonic Acid	
81	12.64	+800	Citric Acid	
81	16.44	+60	Sucrose	
92 FA	9.31	-95	Dodecanoic Acid (12:0)	
92 FA	10.28	-90	Myristic Acid (14:0)	
92 FA	11.20	+500	Hexadecenoic Acid (16:1)	
92 FA	11.96	+200	Oleic Acid (18:1)	
92	10.68	+700	Malic Acid	
92	11.63	+300	Ribonic Acid; Gamma- lactone	
92	12.33	+300	Phosphoric Acid	
92	12.65	-1400	Citric Acid	
92	12.93	+500	Quinic Aci	
92	14.12	+800	Inositol	
50	11.0	New		
50	11.7	New		

[0311] A 3 mm tungsten carbide ball bearing was placed into each well of a 96-well deep well block and 300 μ l of grinding buffer (2 mM NaOH, 1 mM PMSF, 10 mM beta-mercaptoethanol, and deuterium-labeled compounds) was added to each well. A 13 mm circle (~20 mg) leaf disc plug from ~4 week old Nicotiana benthamiana (2 week post-inoculation) apical leaves were placed into the 96-well microtiter deepwell plate. The plate was tightly sealed and placed on a mechanical shaker (paint mixer, up to four at a time) for 2 min, then rotated 180° and shaken for an additional 2 min. Subsequently, the samples were spun for 10 min at 3200 RPM in a refrigerated (15° C.) centrifuge equipped for microtiter plates. Following centrifugation, the 96-well plate containing the homogenized samples was placed on a TECAN GENESIS RSP 200 (TECAN, Research Triangle Park, N.C.) liquid handler/robotics system. Both Logic and Gemini software were used to control the TECAN liquid handler. Approximately 200 μ l was transferred to a pre-conditioned (1 ml MeOH followed by 1 ml of distilled deionized H₂O) Waters 96-well Oasis HLB solid phase extraction (SPE) plate by the TECAN liquid handler for metabolite analysis by GC/MS. The Waters Extraction Plate Manifold Kit and a vacuum not greater than 5 mm Hg was used to aspirate plant samples from SPE plate into a waste reservoir. The SPE plate was then washed with 1 ml of 5% MeOH in H₂O by aspirating into waste reservoir and compounds eluted from SP resin with 350 μ l of MeOH into a 96-well collection plate. Samples were then transferred to GC autosampler vials, capped and stored in the freezer at -80° C. for metabolite analysis.

[0312] XIII. Protein Profiling by MALDI-TOF

[0313] Approximately 14 days post-inoculation, 960 different *N. benthamiana* leaf plugs transfected with encapsidated virion from a GENEWARE® expression library from growth rooms and 38 from *N. benthamiana* infected in Owensboro, Ky. were collected and the soluble proteins extracted with a high throughput micro-extraction technique described below. An aliquot of this solution was automatically diluted with matrix by a liquid handler in preparation for analysis by MALDI-TOF mass spectrometry for proteins.

[0314] A. Sample Preparation by High Throughput Micro-Extraction:

[0315] A 3 mm tungsten carbide ball bearing was placed into each well of a 96-well deep well block and 300 μ l of grinding buffer (2 mM NaOH, 1 mM PMSF, 10 mM beta-mercaptoethanol, and deuterium-labeled compounds-GC/MS analysis) was added to each well. A 13 mm circle (~20 mg) leaf disc plug from ~4 week old Nicotiana benthamiana (2 week post-inoculation) apical leaves were placed into the 96-well microtiter deepwell plate. The plate was tightly sealed and placed on a mechanical shaker (paint mixer, up to four at a time) for 2 min, then rotated 180° and shaken for an additional 2 min. Subsequently, the samples were spun for 10 min at 3200 RPM in a refrigerated (15° C.) centrifuge equipped for microtiter plates. Following centrifugation, the 96-well plate containing the homogenized samples was placed on a TECAN GENESIS RSP 200 (TECAN, Research Triangle Park, N.C.) liquid handler/ robotics system. Both Logic and Gemini software were used to control the TECAN liquid handler. Samples were diluted by the TECAN liquid handler in a round bottom 96-well plate for MALDI-TOF analysis by adding 18 μ l of sinapinic acid matrix and 2 μ l of plant extract to each well. Samples were mixed well by aspirating/dispensing 10 µl volumes five times. A 2 μ l aliquot of each sample was spotted onto a 100 sample MALDI plate. In addition, a 5.0 μ l aliquot of each sample was transferred to a 96-well microtiter plate for PCR and/or MALDI backup analysis and stored at -80° C. Two plant trays containing 96 individually infected each were extracted each day for 5 days.

[0316] B. MALDI-TOF Mass Spectrometry Analysis:

[0317] An aliquot of the homogenized plant samples were diluted 1:10 with sinapinic acid (Aldrich, Milwaukee, Wis.) matrix, 2 µl applied to a stainless steel MALDI plate surface and allowed to air dry for analysis. The sinapinic acid was prepared at a concentration of 10 mg/ml in 0.1% TFA/ acetonitrile (70/30) by volume. MALDI-TOF mass spectra were obtained with a PerSeptive Biosystems Voyager DE-PRO operated in the linear mode. A pulsed nitrogen laser operating at 337 nm was used in the delayed extraction mode for ionization. An acceleration voltage of 25 kV with a 90% grid voltage and a 0.1% guide wire voltage was used. Approximately 150 scans were acquired and averaged over the mass range of 2000-156,000 Da. with a low mass gate of 2000. Ion source and mirror pressures were approximately 2.2×10^{-7} and 8×10^{-8} Torr, respectively. All spectra were mass calibrated with a single-point fit using horse apomyoglobin (16,952 Da).

[0318] C. Results:

[0319] This study describes a method that was developed using the high-throughout capabilities of MALDI-TOF MS to detect changes in total protein profiles of crude plant extracts derived from a GENEWARE® cDNA library. As many as 192 samples per day were extracted and analyzed for protein profiling using MALDI-TOF mass spectrometry. In addition, the method has been optimized in house for

detection of a wide range of protein masses from one MALDI-TOF scan. More than 50 proteins were routinely detected in a MALDI profile spectrum ranging from approx. 3,000 to 110,000 Da. In addition to the coat protein (~17,500 Da), both small (~14,500 Da) and large (~52,750 Da) subunits of RuDP carboxylase were routinely detected in the plant samples. Several other proteins were common to most of the plants analyzed. The most abundant proteins were observed at around 3,386, 3,970, 4,408, 5,230, 7,280 (doubly charged ion for small sub-unit of RuDP carboxylase), 8,334, 9,350, 10,450 (most abundant protein overall), 14,020, 18,006, 19,628, 20,286, 21,173, 24,014, 25,124 and 29,140 (dimer of small sub-unit) daltons. A series of less abundant proteins were also detected. Up-regulated or novel proteins were detected in 17.3% of the 960 spectra that were analyzed. This data was entered into the LIMS database.

[0320] XIV. ABRC Library Construction in GENEWARE Expression Vectors

[0321] Expressed sequence tag (EST) clones were obtained from the Arabidopsis Biological Resource Center (ABRC; The Ohio State University, Columbus, Ohio 43210). These clones originated from Michigan State University (from the labs of Dr. Thomas Newman of the DOE Plant Research Laboratory and Dr. Chris Somerville, Carnegie Institution of Washington) and from the Centre National de la Recherche Scientifique Project (CNRS project; donated by the Groupement De Recherche 1003, Centre National de la Recherche Scientifique, Dr. Bernard Lescure and colleagues). The clones were derived from cDNA libraries isolated from various tissues of *Arabidopsis thaliana* var Columbia. A clone set of 11,982 clones was received as glycerol stocks arrayed in 96 well plates, each with an ABRC identifier and associated EST sequence.

[0322] An ORF finding algorithm was performed on the EST clone set to find potential full-length genes. Approximately 3,200 full-length genes were found and used to make GENEWARE constructs in the sense orientation. Five thousand of the remaining clones (not full-length) were used to make GENEWARE constructs in the antisense orientation.

[0323] Full-length clones used to make constructs in the sense orientation were grown and DNA was isolated using Qiagen (Qiagen Inc., Valencia, Calif. 91355) mini-preps. Each clone was digested with NotI and Sse 8387 eight base pair enzymes. The resultant fragments were individually isolated and then combined. The combined fragments were ligated into pGTN P/N vector (with polylinker extending from PstI to NotI -5' to 3'). For each set of 96 original clones approximately 192 colonies were picked from the pooled GENEWARE ligations, grown until confluent in deep-well 96-well plates, DNA prepped and sequenced. The ESTs matching the ABRC data was bioinformatically checked by BLAST and a list of missing clones was generated. Pools of clones found to be missing were prepared and subjected to the same process. The entire process resulted in greater than 3,000 full-length sense clones.

[0324] The negative sense clones were processed in the same manner, but ligated into pGTN N/P vector (with polylinker extending from NotI to PstI -5' to 3'). For each set of 96 original clones approximately 192 colonies were picked from the pooled geneware ligations and DNA prepped. The DNA from the GENEWARE ligations was subjected to RFLP analysis using TaqI 4 base cutter. Novel

patterns were identified for each set. The RFLP method was applied and only applicable for comparison within a single ABRC plate. This procedure resulted in greater than 6,000 negative sense clones.

[0325] The identified clones were re-arrayed, transcribed, encapsidated and used to inoculate plants.

[0326] XV. Inoculation of Plants

[0327] A. Plant Growth.

[0328] N. benthamiana seeds were sown in 6.5 cm pots filled with Redi-earth medium (Scotts) that had been prewetted with fertilizer solution (prepared by mixing 147 kg Peters Excel 15-5-15 Cal-Mag (The Scotts Company, Marysville Ohio), 68 kg Peters Excel 15-0-0 Cal-Lite (15% Ca), and 45 kg Peters Excel 10-0-0 MagNitrate (10% Mg) in hot tap water to 596 liters total volume and then injecting this concentrate into irrigation water using an injection system (H. E. Anderson, Muskogee Okla.), at a ratio of 200:1). Seeded pots were placed in the greenhouse for 1 d, transferred to a germination chamber, set to 27° C., for 2 d (Carolina Greenhouses, Kinston, N.C.), and then returned to the greenhouse. Shade curtains (33% transmittance) were used to reduce solar intensity in the greenhouse and artificial lighting, a 1:1 mixture of metal halide and high pressure sodium lamps (Sylvania) that delivered an irradiance of approximately 220 μ mol m²s⁻¹, was used to extend day length to 16 h and to supplement solar radiation on overcast days. Evaporative cooling and steam heat were used to regulate greenhouse temperature, maintaining a daytime set point of 27° C. and a nighttime set point of 22° C. At approximately 7 days post sowing (dps), seedlings were thinned to one seedling per pot and at 17 to 21 dps, the pots were spaced farther apart to accommodate plant growth. Plants were watered with Hoagland nutrient solution as required. Following inoculation, waste irrigation water was collected and treated with 0.5% sodium hypochlorite for 10 minutes to neutralize any viral contamination before discharging into the municipal sewer.

[0329] B. Innoculation.

[0330] For each GENEWARETM clone, 180 μ L of inoculum was prepared by combining equal volumes of encapsidated RNA transcript and FES buffer (0.1M glycine, 0.06 M K₂HPO₄, 1% sodium pyrophosphate, 1% diatomaceous earth (Sigma), and either 1% silicon carbide (Aldrich), or 1% Bentonite (Sigma)). The inoculum was applied to three greenhouse-grown Nicotiana benthamiana plants at 14 or 17 days post sowing (dps) by distributing it onto the upper surface of one pair of leaves of each plant (30 μ L per leaf). Either the first pair of leaves or the second pair of leaves above the cotyledons was inoculated on 14 or 17 dps plants, respectively. The inoculum was spread across the leaf surface using one of two different procedures. The first procedure utilized a Cleanfoam swab (Texwipe Co, N.J.) to spread the inoculm across the surface of the leaf while the leaf was supported with a plastic pot label (3/4×5 2M/RL, White Thermal Pot Label, United Label). The second implemented a 3" cotton tipped applicator (Calapro Swab, Fisher Scientific) to spread the inoculum and a gloved finger to support the leaf. Following inoculation the plants were misted with deionized water.

[0331] C. Infection.

[0332] At 13 days post inoculation (dpi), the plants were examined visually and a numerical score was assigned to each plant to indicate the extent of viral infection symptoms. 0=no infection, 1=possible infection, 2=infection symptoms limited to leaves<50-75% fully expanded, 3=typical infection, 4=atypically severe infection, often accompanied by moderate to severe wilting and/or necrosis.

[0333] XVI: Phenotypic Evaluation

[0334] At 13 dpi plants were examined and in cases where a plant's visual phenotype deviated substantially from the phenotypes of control plants, a controlled vocabulary utilizing a five-part phrase was used to describe the plants. Phrase: plant region/sub-part/modifier (optional)/symptom/ severity. Plant regions: sink leaves (the upper region of the plant considered to be primarily phloem sink tissue at the time of evaluation), source leaves (expanded, fully-infected leaves considered to be phloem source tissue at the time of evaluation), bypassed leaves (leaves [three and four] that display little or no infection symptoms), inoculated leaves (leaves one and two), stem. Subparts: blade, entire, flower, foci, intervein, leaf, lower, major vein, margin, minor vein, node, petiole, shoot apex, upper, vein, viral path. Modifiers: apical, associated, banded, basal, blotchy, bright, central, crinkled, dark, epinastic, flecked, glossy, gray, hyponastic, increased, intermittent, large-spotted, light, light-colored, light-green, mottled, narrowed, orange, patchy, patterned, radial, reduced, ringspot, small-spotted, smooth, spotted, streaked, subtending, uniform, unusual, white. Symptoms: bleaching, chlorosis, color, contortion, corrugation, curling, dark green, elongation, etching, hyperbranching, mild symptoms, necrosis, patterning, recovery, stunting, texture, trichomes, wilting. Severity: 1-extremely mild/trace, 2-mild symptom (<30% of subpart affected), 3-moderate symptom (30%-70% of subpart affected), 4-severe symptom (>70% of subpart affected). Based on the symptoms a phenotypic hit value (PHV) and a herbicide hit value (HHV) were assigned to each plant phenotyped. Phenotype Hit Value: 1-no predicted value; do not request for repeat analysis, 2-of uncertain value, 3-of potential value; strong phenotype, 4-highly unusual phenotype. Herbicide Hit Value: 1-no predicted value; do not request for repeat analysis, 2-of uncertain value, 3-moderate chlorosis (especially in apical region) or necrosis, 4-Severe phytotoxicity/herbicide mode of action. Comments were added if additional information was required to complete the plant characterization. Results are presented in Table 8.

TABLE 8

SEQ ID NO	Library	Summary of Visual Phenotype
SEQ ID NO:12	ABRC	Stunting
SEQ ID NO:27	ABRC	Stunting
SEQ ID NO:48	ABRC	Stunting
SEQ ID NO:49	ABRC	Stunting
SEQ ID NO:59	ABRC	Stunting
SEQ ID NO:60	ABRC	Stunting
SEQ ID NO:71	ARAB	Stunting
SEQ ID NO:84	ABRC	Stunting
SEQ ID NO:99	ABRC	Stunting
SEQ ID NO:100	ABRC	Stunting
SEQ ID NO:102	ABRC	Stunting
SEQ ID NO:103	ABRC	Stunting
SEQ ID NO:105	ABRC	Stunting
		-

_	SEQ ID NO	Liotary	Summary of Visual Thenotype
-	SEQ ID NO:106 SEQ ID NO:107 SEQ ID NO:108 SEQ ID NO:109 SEO ID NO:110	ABRC ABRC ABRC ABRC ABRC	Stunting Stunting Stunting Stunting Stunting

[0335] XVII: Metabolic Screens

[0336] A. Sample Generation.

[0337] Individual dwarf tobacco *nicotiana benthamiana*, (Nb) plants were manually transfected with an unique DNA sequence at 14 or 17 days post sowing using the GENEW-ARETM viral vector technology (1). Plants were grown and maintained under greenhouse conditions. At 13 days after infection, an infection rating of 0, 1, 2, 3, or 4 was assigned to each plant. The infection rating documents the degree of infection based on a visual observation. A score of 0 indicates no visual infection. Scores of 1 and 2 indicate varying degrees of partial infection, the plant is either dead or near death. A score of 3 indicates optimum spread of systemic infection.

[0338] Samples were grouped into sets of up to 96 samples per set for inoculation, harvesting and analysis. Each sample set (SDG) included 8 negative control (reference samples), up to 80 unknown (test) samples, and 8 quality control samples.

[0339] B. Harvesting.

[0340] At 14 days after infection, infected leaf tissue, excluding stems and petioles, was harvested from plants with an infection score of 3. Infected tissue was placed in a labeled, 50-milliliter (mL), plastic centrifuge tube containing a tungsten carbide ball approximately 1 cm in diameter. The tube was immediately capped, and dipped in liquid nitrogen for approximately 20 seconds to freeze the sample as quickly as possible to minimize degradation of the sample due to biological processes triggered by the harvesting process. Harvested samples were maintained at -80 C between harvest and analysis. Each sample was assigned a unique identifier, which was used to correlate the plant tissue to the DNA sequence that the plant was transfected with. Each sample set was assigned a unique identifier, which is referred to as the harvest or meta rack ID.

[0341] C. Extraction.

[0342] Prior to analysis, the frozen sample was homogenized by placing the centrifuge tube on a mechanical shaker. The action of the tungsten carbide ball during approximately 30 seconds of vigorous shaking reduced the frozen whole leaf tissue to a finely homogenized frozen powder. Approximately 1 gram of the frozen powder was extracted with 7.5 mL of a solution of isopropanol (IPA-):water 70:30 (v:v) by shaking at room temperature for 30 minutes.

[0343] D. Fractionation.

[0344] A 1200 microliter (μ L) aliquot of the IPA:water extract was partitioned with 1200 μ L of hexane. The hexane

layer was removed to a clean glass container. This hexane extract is referred to as fraction 1 (F1). A 90 μ L aliquot of the hexane extracted IPA:water extract was removed to a clean glass container. This aliquot is referred to as fraction 4 (F4). The remaining hexane extracted IPA:water extract is referred to as fraction 3 (F3). A 200 μ L aliquot of the IPA:water extract was transferred to a clean glass container and referred to as fraction 2 (F2). Each fraction for each sample was assigned a unique aliquot ID (sample name).

[0345] E. Sample Preparation & Data Generation

[0346] Fraction 1:

[0347] The hexane extract was evaporated to dryness under nitrogen at room temperature. The sample containers were sealed and stored at 4 C prior to analysis, if storage was required. Immediately prior to capillary gas chromatographic analysis using flame ionization detection (GC/FID), the F1 residue was reconstituted with 120 μ L of hexane containing pentacosane and hexatriacontane which were used as internal standards for the F1 analyses. The chromatographic data files generated following GC separation and flame ionization detection were named with the fraction 1 aliquot ID for each sample and stored in a folder named after the harvest rack (sample set) ID. **FIG. 1 a** summarizes the GC/FID parameters used to analyze fraction 1 samples.

[0348] Fraction 2:

[0349] The F2 aliquot was evaporated to dryness under nitrogen at room temperature and reconstituted in heptane containing 2 internal standards, C11:0 and C24:0. In general, fraction 2 is designed to analyze esterified fatty acids, such as phospholipids, triacylglycerides, and thioesters. In order to analyze these compounds by GC/FID, they were transmethylated to their respective methyl esters by addition of sodium methoxide in methanol and heat. Excess reagent was quenched by the addition of a small amount of water, which results in phase separation. The fatty acid methyl esters (FAMEs) were contained in the organic phase. **FIG.** 1*b* summarizes the GC/FID parameters used to analyze fraction 1 samples.

[0350] Fraction 3:

[0351] The F3 aliquot was evaporated to dryness under nitrogen at 40 C. In general, the metabolites in this fraction are highly polar and water-soluble. In order to analyze these compounds by GC/FID, the polar functional groups on these compounds were silvlated through a 2-step derivatization process. Initially, the residue was reconstituted with 400 μ L of pyridine containing hydroxylamine hydrochloride (25 mg/ml) and the internal standard, n-octyl-β-D-glucopyranoside (OXIME solution). The derivatization was completed by the addition of 400 μ L of the commercially available reagent (N,O-bis[Trimethylsily] trifluoroacetamide)+1% Trimethylchlorosilane (BSTFA+1% TMCS). The chromatographic data files generated following GC separation and flame ionization detection were named with the fraction 3 aliquot ID for each sample and stored in a folder named after the harvest rack (sample set) ID. FIG. 1c summarizes the GC/FID parameters used to analyze fraction 1 samples.

[0352] Fraction 4:

[0353] The F4 aliquot was diluted with 90 μ L of distilled water and 20 μ L of an 0.1 N hydrochloric acid solution containing norvaline and sarcosine, which are amino acids

that are used as internal standards for the amino acids analysis. Immediately prior to high performance liquid chromatographic analysis using fluorescence detection (HPLC/FLD), the amino acids in F4 are mixed in the HPLC injector at room temperature with buffered orthophtaldehyde solution, which derivatizes primary amino acids, followed by fluorenyl methyl chloroformate, which derivatizes secondary amino acids. Following HPLC separation and fluorescence detection, chromatographic data files were generated for each sample, named with a sequential number which can be tracked back to the F4 aliquot ID, and stored in a folder named after the harvest rack (sample set) ID. **FIG.** 1*d* summarizes the GC/FID parameters used to analyze fraction 1 samples.

[0354] F. Data Analysis & Hit Detection.

[0355] Two complementary methods were used to identify modifications in the metabolic profile of test samples from reference samples. These data analysis methods are called automated data analysis (ADA) and quantitative data analysis. Each fraction from each sample was analyzed by one or both of these methods to identify hits. If either method identified a fraction as a hit, the sample was called a hit for that fraction. Therefore a sample could be a hit for 1 through 4 fractions.

[0356] ADA employs a qualitative pattern recognition approach using ABNORM (U.S. Pat. No. 5,592,402), which is a proprietary software utility of the Dow Chemical Company. ADA was performed on chromatograms from all 4 fractions. The ADA process developed a statistical model from chromatograms that ideally depict unaltered (reference) metabolic profiles. This model was then used to identify test sample chromatograms that contain statistically significant differences from the normal (control) chromatograms. Updated models for each fraction were generated for each sample set. Chromatograms identified as hits by ADA, were manually reviewed and the data quality visually verified.

[0357] Quantitative data analysis is based on individual peak areas. Quantitative data analysis was applied to specific compounds of interest in fraction 2, fatty acids, and fraction 4, amino acids. The peak areas corresponding to these compounds in these fractions were generated. For fraction 2, the relative percent of the peak areas for the compounds in Table 9 were calculated for each sample. The average (\bar{x}) and standard deviation (STD) of the relative % of the peak areas for the individual compounds were calculated from the reference sample chromatograms analyzed within the sample set. The average and STD were used to calculate a range for each compound. Depending on the compound, this range was typically \bar{x} +/-3 or 5 STDs. If the relative percent of the peak area from an unknown was outside this range, the compound was considered to be significantly different from the 'normal' level and the sample was identified as a hit for F2. For fraction 4, the concentration, in micrograms/gram was calculated for each of the amino acids listed in Table 9, from calibration standards analyzed at the same time as the test samples. The amino acid concentrations from reference samples were used to calculate the acceptable range from the \bar{x} and STD for each amino acid. If the amino acid concentration for an unknown falls outside this range, the amino acid was considered to be different from normal and sample was identified as a hit for F4.\

TABLE 9

Tobacco Metabolites Monito Quantitativ		ions 2 and 4 by		
Fraction 2 (Fatty Acids) Fraction 2				
undecanoic acid methyl ester*	C11:0	Aspartic Acid	ASP	
Pentadecanoic acid methyl ester**	C15:0	Glutamic Acid	GLU	
Pentadecanoic acid ethyl ester**	C15:0	Serine	SER	
palmitic acid methyl ester	C16:0	Histidine	HIS	
palmitoleic acid methyl ester	C16:1	Glycine	GLY	
iso methylpentadecanoic acid methyl ester	C16:0:Me	Threonine	THR	
palmitoleic acid methyl ester	C16:2	Alanine	ALA	
palmitolenic acid methyl ester	C16:3	Arginine	ARG	
iso methylhexadecanoic acid methyl ester	C17:0Me	Tyrosine	TYR	
Stearic acid methyl ester	C18:0	Cystine	CY2	
Oleic acid methyl ester	C18:1	Valine	VAL	
Linoleic acid methyl ester	C18:2	Methionine	MET	
Linolenic acid methyl ester	C18:3	Norvaline*	NVA	
Arachidic acid methyl ester	C20:0	Tryptohane	TRP	
Lignoceric acid methyl ester*	C24:0	Phenylalanine	PHE	
		Isoleucine	ILE	
		Leucine	LEU	
		Lysine	LYS	
		Sarcosine*	SAR	
		Proline	PRO	

*Internal Standard **Surrogate Standard

[0358] Shipping Hits.

[0359] Any F1, F2, or F3 fractions identified as hits by ADA or quantitative analysis, and the most typical null for each fraction for each sample set as identified by ADA, were sent to the Function Discovery Laboratory (see Example 20) for structural characterization of the specific compounds identified. Samples were sealed, packaged on dry ice and shipped for overnight delivery.

[0360] XVIII: Identification of Metabolic Changes

[0361] This Example describes the identification of the chemical nature of genetic modifications made in tobacco plants using GENEWARE viral vector technology. The protocols involved the use of gas chromatography/mass spectrometry (GC/MS) for the analyses of three primary fractions obtained from extraction and fractionation processes.

[0362] A. Methods.

[0363] Major instruments and accessories used included Bioinformatics computer programs, mass spectral libraries, Biotech databases, Nautilus LIMS system (BLIMS; Dow), Biotech Database (eBRAD; Dow), HP Model 6890 capillary Gas Chromatograph (GC; Agilent Technologies), HP Model 5973 Mass Selective Detector (MSD; Agilent Technologies), Auto Sampler and Sample Preparation Station (Leap Technologies), Large Volume Injector system (APEX), Ultra Freezer (Revco), and model LS1006 Barcode Reader (Symbol Technologies).

[0364] Samples and corresponding References (also referred to as controls or nulls) were shipped via overnight mail. Samples were removed from the shipping container, inspected for damage, and then placed in a freezer until analysis by GC/MS.

[0365] Samples were received in vials or in titer plates with a bar-coded titer plate (TP) number, also referred to as a Rack Identification number that is used to track the sample in the BLIMS system. The barcode number is used by the FDL to extract from BLIMS pertinent information from ADA (Automated chromatographic pattern recognition Data Analysis) HIT reports and/or QUANT (a quantitative data analysis approach that makes use of individual peak areas of select peaks corresponding to specific compounds of interest in the fatty acid Fraction 2) HIT reports generated by the Metabolic Screening Laboratory. The information in these reports includes the well position of the respective HITs (Samples), the corresponding well position of the Reference, and other pertinent information, such as, aliquot identification. This information is used to generate ChemStation and Leap sequences for FDL analyses.

[0366] Samples were sequenced for analysis in the following order:

TABLE 10

Analysis Order			
	Solvent Blank Instrument Performance Standard Samples and Associated Reference		
	· · ·		
	Performance Standard Solvent Blank		

[0367] Samples were analyzed on GC/MS systems using the following procedures. Fraction 1 samples were shipped dry and required a hexane reconstitution step. Fraction 2 and Fraction 3 samples were analyzed as received. Internal standards were added to the samples prior to analysis.

[0368] B. Fraction 1 Analysis.

[0369] The name of the GC/MS method used is BION-EUTx (where x is a revision number of the core GC/MS method). The method is retention-time locked to the retention time of pentacosane, an internal standard, using the ChemStation RT Locking algorithm.

Internal Standard(s) Pentacosane Hexatriacontane Chromatography	
Column:	J & W DB-5MS
Mode: constant flow	50 M × 0.320 mm × 0.25 μ m film
	Flow: 2.0 mL/min
Oven:	Detector: MSD Outlet psi: vacuum 40° C. for 2.0 min 20° C./min to 350° C., hold 15.0 min
	Equilibration time: 1 min
Inlet:	Mode: split Inj Temp: 250° C.
LEAP Injector:	Split ratio: 50:1 Gas Type: Helium
Injector:	Inj volume: optimized to pentacosane peak intensity (typically 20 $\mu \rm L)$

	-continued
APEX Injector Method Name: Modes:	Sample pumps: 2 Wash solvent A: Hexane Wash solvent B: Acetone Preinj Solvent A washes: 2 Postinj Solvent B washes: 2 Postinj Solvent B washes: 2 Postinj Solvent B washes: 2 BIONEUTx (where x is a revision number of the core APEX method). Initial: Standby (GC Split) Splitless: (Purge Off) 0.5 min GC Split: (Standby) 4 min
Temps: 50° C. for 0.0 min. Mass Spectrometer Scan: 35–800	ProSep Split: (Flow Select) 23 min 300° C./min to 350° C., hold for 31.5 min Da at sampling rate 2 (1.96 scans/sec) Solvent delay: 4.0 min
Detector:	EM absolute: False EM offset: 0
Temps:	Transfer line: 280° C. Ion source: 150° C. MS Source: 230° C.

[0370] C. Fraction 2 Analysis:

[0371] The name of the GC/MS method used is BIO-FAMEx (where x is a revision number of the core GC/MS method). The method is retention-time locked to RT of undecanoic acid, methyl ester, an internal standard, using the ChemStation RT Locking algorithm.

Internal Standard(s)	
Undecanoic acid, methyl ester	
Tetracosanoic acid, methyl ester	
Chromatography	
Column:	J & W DB-23 FAME
	60 M × 0.250 mm × 0.15 μ m film
	Mode: constant flow
	Flow: 2.0 mL/min
	Detector: MSD
0	Outlet psi: vacuum
Oven:	50° C. for 2.0 min
	20° C./min to 240° C., hold 10.0 min
T 1 4	Equilibration time: 1 min
Inlet:	Mode: split
	Inj Temp: 240° C.
	Split ratio: 50:1
	Gas Type: Helium
LEAP Injector:	
Injector:	Inj volume: optimized to undecanoic
	acid, methyl ester peak intensity
	(Typically 10 µL)
	Sample pumps: 2
	Wash solvent A: Methanol
	Wash solvent B: Methanol
	Preinj Solvent A washes: 2
	Preinj Solvent B washes: 2
	Postinj Solvent A washes: 2
	Postinj Solvent B washes: 2
APEX Injector	
Method Name:	BIOFAMEx (where x is a revision
	number of the core APEX method).

-continued
Initial: GC Split
Splitless: 0.5 min
GC Split: 4 min
21 min
60° C. for 0.5 min.
300° C./min to 250° C., hold for 20 min
300° C./min to 260° C., hold for 5 min
Da at sampling rate 2 (1.96 scans/sec)
Solvent delay: 4.5 min
EM absolute: False
Transfer line: 200° C.
Ion source: 150° C.
MS Source: 230° C.

[0372] D. Fraction 3 Analysis.

[0373] The name of the GC/MS method used is BIO-AQUAx (where x is a revision number of the core GC/MS method). Method is retention-time locked to the RT of n-Octyl- β -D-Glucopyranoside, an internal standard, using the ChemStation RT Locking algorithm.

Internal Standard(s)	
n-Octyl-B-D-Glucopyranoside	
Chromatography	
Column:	Chrompack 7454 CP-SIL 8
	60 M \times 0.320 mm \times 0.25 μ m film
	Mode: constant flow
	Flow: 2.0 mL/min
	Detector: MSD
	Outlet psi: vacuum
Oven:	40° C. for 2.0 min
	20° C./min to 350° C., hold 10.0 min
	Equilibration time: 1 min
Inlet: Mode: split	•
	Inj Temp: 250° C.
	Split ratio: 50:1
	Gas Type: Helium
LEAP Injector:	
Injector:	Inj volume: Optimized to n-Octyl-β-D-
	Glucopyranoside peak intensity
(Typically 2.5 μ L)	
	Sample pumps: 2
	Wash solvent A: Hexane
	Wash solvent B: Acetone
	Preinj Solvent A washes: 2
	Preinj Solvent B washes: 2
	Postinj Solvent A washes: 2
	Postinj Solvent B washes: 2
APEX Injector	
Method Name:	BIQAQUAx (where x is a revision
	number of the core APEX method).
Modes:	Initial: GC Split
	Splitless: 0.5 min
	GC Split: 4 min
	ProSep Split: 20 min
Temps:	60° C. for 0.5 min.
	300° C./min to 350° C., hold for 21.1 min
Mass Spectrometer	
Scan: 35-800	Da at sampling rate 2 (1.96 scans/sec)
_	Solvent delay: 4.0 min
Detector:	EM absolute: False
EM offset: 0	T (1) 2000 C
Temps:	Transfer line: 280° C.
	Ion source: 150° C.
	MS Source: 230° C.

[0374] E. Performance Standard:

[0375] Two mixtures were used as instrument performance standards. One standard was run with Fraction 1 and 3 samples and the second was run with Fraction 2 samples. Below is the composition of the standards as well as approximate retention time values observed when run under the GC/MS conditions previously described. These retention time values are subject to change depending upon specific instrument and chromatographic conditions.

TABLE 11

Time Compound	
6.25 dimethyl malonate	
7.25 dimethyl succinate	
8.15 dimethyl glutarate	
8.98 dimethyl adipate	
11.06 dimethyl azelate	
11.42 hexadecane	
11.70 dimethyl sebacate	
13.57 eicosane	
15.36 tetracosane	
16.88 octacosane	
18.26 dotriacontane	
19.95 hexatriacontane	

[0376]

TABLE 12

Fraction 2 Performance Standard				
Time	Compound			
8.82	undecanoic acid, methyl ester			
9.32	dodecanoic acid, methyl ester			
10.24	tetradecanoic acid, methyl ester			
11.07	hexadecanoic acid, methyl ester			
11.84	octadecanoic acid, methyl ester			
11.90	oleic acid, methyl ester			
12.14	linoleic acid, methyl ester			
12.39	linolenic acid, methyl ester			
12.60	eicosanoic acid, methyl ester			
13.42	docosanoic acid, methyl ester			

[0377] F. Data Analysis.

[0378] Sample and Reference data sets were processed using the Bioinformatics computer program Maxwell. The principal elements of the program are 1) Data Reduction, 2) two-dimensional Peak Matching, 3) Quantitative Peak Differentiation (Determination of Relative Quantitative Change), 4) Peak Identification, 5) Data Sorting, and 6) Customized Reporting.

[0379] The program queries the user for the filenames of the Reference data set and Sample data set(s) to compare against the Reference. A complete listing of user inputs with example input is shown below.

TABLE 13

Bioinformatics Analysis					
USER QUERY	EXAMPLE USER INPUT				
Operator Name	M. Maxwell				
Total number of data files to process	5				
Which Fraction	3				
Reference (Control) File Name	AAPR0020.D				
Process a specific RT Range	Y				
Specific RT range	6.5-23				
Internal Standard Retention Time	14.902				
+/- variation in Internal Std. RT	.004				
Variation in peak RI, ChemStation	.005				
Percent variation in peak RI, Biotech	.010				
Database					
Threshold for determining Area % change	60				
Spectral Matching Value (Threshold MS-	.95				
XCR for peaks to be a match)					
Percent to determine LOP-PM* Value	1				
Percent to determine LOP-SRT** Value	3				
Quality Level for Library (Library match)	80				
Subtract Background	Y				
Time Range for Background	21.5-22.6				
SHORT SUMMARY (y/n , $y = no$	Y				
chromatograms)					

*LOP-PM - Limit of Processing for Peak Matching

**LOP-SRT - Limit of Processing for Sorting

[0380] The program integrates the Total Ion Chromatogram (TIC) of the data sets using Agilent Technologies HP ChemStation integrator parameters determined by the analyst. The corresponding raw peak areas are then normalized to the respective Internal Standard peak area. It should be noted that before the normalization is performed, the program chromatographically and spectrally identifies the Internal Standard peak. Should the identification of the Internal Standard not meet established criteria for a given Fraction, then the data set will not be further processed and it will be flagged for analyst intervention.

[0381] Peak tables from the Reference and each Sample were generated. The peak tables are comprised of retention time (RT), retention index (RI)—the retention time relative to the Internal Standard RT, raw peak areas, peak areas normalized to the Internal Standard, and other pertinent information.

[0382] The first of two filtering criteria, established by the analyst was then invoked and must be met before a peak is further processed. The criterion is based upon a peak's normalized area. All normalized peaks having values below the Limit of Processing for Peak Matching (LOP-PM), were considered to be "background". These "peaks" were not carried forth for any type of mathematical calculation or spectral comparison.

[0383] In the initial peak-matching step, the Sample peak table was compared to the Reference peak table and peaks between the two were paired based upon their respective RI values matching one another (within a given variable window). The next step in the peak matching routine utilized mass spectral data. Sample and Reference peaks that have been chromatographically matched were then compared spectrally. The spectral matching was performed using a mass spectral cross-correlation algorithm within the Agilent Technologies HP ChemStation software. The cross-correlation algorithm generates an equivalence value based upon

spectral "fit" that was used to determine whether the chromatographically matched peaks are spectrally similar or not. This equivalence value is referred to as the MS-XCR value and must meet or exceed a predetermined value for a pair of peaks to be "MATCHED," which means they appear to be the same compound in both the Reference and the Sample. The MS-XCR value can also be used to judge peak purity. This two-dimensional peak matching process was repeated until all potential peak matches were processed. At the end of the process, peaks are categorized into two categories, MATCHED and UNMATCHED.

[0384] A second filtering criterion was next invoked, again based upon the normalized area of the MATCHED or UNMATCHED peak. For a peak to be reported and further processed, its normalized area must meet or exceed the predetermined Limit of Processing for Sorting (LOP-SRT).

[0385] Peaks that are UNMATCHED are immediately flagged as different. UNMATCHED peaks are of two types. There are those that are reported in the Reference but appear to be absent in the Sample (based upon criteria for quantitation and reporting). These peaks were designated in the Analyst Report with a percent change of "-100 percent" and the description "UNMATCHED IN SAMPLE." The second types of peaks are those that were not reported in the Reference (again, based upon criteria for quantitation and reporting) but were reported in the Sample, thus appearing to be "new" peaks. These peaks were designated in the Analyst Report with a percent change of "100 percent" and the description "NEW PEAK UNMATCHED IN NULL."

[0386] MATCHED peaks were processed further for relative quantitative differentiation. This quantitative differentiation is expressed as a percent change of the Sample peak area relative to the area of the Reference peak. A predetermined threshold for change must be observed for the change to be determined biochemical and statistically significant. The change threshold is based upon previously observed biological and analytical variability factors. Only changes above the threshold for change were reported.

[0387] Peaks were then processed through the peak identification process as follows. The mass spectra of the peaks were first searched against mass spectral plant metabolite libraries. The equivalence value assigned to the library match was used as an indication of a proper identification.

[0388] To provide additional confirmation to the identity of a peak, or to suggest other possibilities, library hits were searched further against a Biotechnology database. The Biotechnology database is based on the Access database program from Accelrys (formerly Synopsis) and utilizes Accord for Access (also available from Accelrys) to incorporate chemical structures into the database.

[0389] The Chemical Abstract Services (CAS) number of the compound from the library was searched against those contained in the database. If a match was found, the CAS number in the database was then correlated to the data acquisition method for that record. If the method was matched, the program then compared the retention index (RI), in the Peak Table, of the component against the value contained in the database for that given method. Should the RI's match (within a given window of variability) then the peak identity was given a high degree of certainty. Components in the Sample that are not identified by this process were assigned a unique identifier based upon Fraction Number and RI (example: F1-U0.555). The unique identifier was used to track unknown components. The program then sorts the data and generates an Analyst Report.

[0390] An Analyst Report is an interim report consisting of PBM algorithm match quality value (equivalence value), RT, Normalized Peak Area, RI (Sample), RI (database) Peak Identification status [peak identity of high certainty (peaks were identified by the program based on the pre-established criteria) or criteria not met (program did not positively identify the component)], Component Name, CAS Number, Mass Spectral Library (containing spectrum most closely matched to that of the component), Unknown ID (unique identifier used to track unidentified components), MS-XCR Change, Notes Relative % (MATCHED value. UNMATCHED), and other miscellaneous information. The Analyst Report was reviewed manually by the analyst who determined what further analysis was necessary. The analyst also generated a modified report, for further processing by the program, by editing the Analyst Report accordingly.

[0391] For Fractions 2 and 3, derivatization procedures were performed prior to analysis to make the certain components more amenable to gas chromatography. Thus, the compound names in the modified analyst report (MAR) were those of the derivatives. To accurately reflect the true components of these fractions, the MAR was further processed using information contained in an additional database. This database cross-references the observed derivatized compound to that of the original, underivatized "parent" compound by way of their respective CAS numbers and replaces derivatives with parent names and information for the final report. In addition, any unidentified components were assigned a "999999-99-9" CAS number.

[0392] The Modified Analyst Report also contains a HIT Score of 0, 1, or 2. The value is assigned by the analyst to the data set of the Sample aliquot based on the following criteria:

[0393] 0 No FDL data on Sample

[0394] 1 FDL data collected; Sample not FDL HIT

[0395] 2 FDL data collected; Sample is FDL HIT

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[0396] An FDL HIT is defined as a reportable percent change (modification) observed in a Sample relative to Reference in a component of biochemical significance.

[0397] An electronic copy of the final report is entered into the Nautilus LIMS system (BLIMS) and subsequently into eBRAD (Biotech database). The program also generated a hardcopy of the pinpointed TIC and the respective mass spectrum of each component that was reported to have changed.

[0398] "NQ" and "NEW" are two terms used in the final report. Both terms refer to UNMATCHED peaks whose percent changes cannot be reported in a numerically quantitative fashion. These terms are defined as follows:

[0399] "NQ" is used in the case where there was a peak reported in the Reference for which there was no match in the Sample (either because there was no peak in the Sample or, if there was, the area of the peak did not satisfy the Limit of Processing for Peak Matching). The percent change designation of "-100%" used in the Analyst report is replaced with "NQ".

[0400] "NEW" is used in those situations where a peak was reported in the Sample but for which there was no corresponding match in the Reference (either because there was no peak in the Reference or, if there was, the area of the peak did not satisfy the Limit of Processing for Peak Matching). For these situations, the percent change designation of "100%" used in the Analyst Report is replaced with "NEW". The designation of "NEW" in the final report to a component that is present in the Sample but not in the Reference was necessary to eliminate any ambiguity with the appearance of "100%" for MATCHED peaks. A "100%" designation in the final report exclusively refers to a component with modification that doubled in the Sample relative to the Reference.

[0401] G. Results.

[0402] The results of the metabolic screening revealed that transfection with 55 of the inserts resulted in measurable metabolic changes.

SEQUENCE LISTING

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Dec. 19, 2002

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107

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1. A method of creating a transfected or transgenic plant chosen from the group consisting of ornamental, horticultural, forestry, medicinal or Nicotiana sp. plants, exhibiting a dwarf phenotype comprising: expressing in the plant the DNA identified by a polynucleotide sequence chosen from the group consisting of SEQ. ID NO: 1-122 or the mRNA encoded by the DNA identified by a polynucleotide sequence chosen from the group consisting of SEQ. ID NO: 1-122:

2. A method of creating a transfected or transgenic plant chosen from the group consisting of ornamental, horticultural, forestry, medicinal or Nicotiana sp. plants, exhibiting a dwarf phenotype comprising the steps of:

- (a) providing a viral inoculum capable of infecting a plant comprising the DNA identified by a polynucleotide sequence chosen from the group of SEQ. ID NO: 1-122 or the mRNA encoded by the DNA identified by a polynucleotide sequence chosen from the group of SEQ. ID NO: 1-122;
- (b) applying said viral inoculum to a plant;
- whereby the plant is infected and the DNA or the mRNA is expressed in the plant.

3. The method of claims 1 or 2 wherein the plant is turfgrass.

4. The method of claims 1 or 2 wherein the plant is fir tree.

5. A transfected or transgenic plant chosen from the group consisting of ornamental, horticultural, forestry, medicinal or Nicotiana sp. plants, exhibiting a dwarf phenotype made by the method comprising: expressing in the plant the DNA identified by a polynucleotide sequence chosen from the group consisting of SEQ. ID NO: 1-122 or the mRNA encoded by the DNA identified by a polynucleotide sequence chosen from the group consisting of SEQ. ID NO: 1-122 or the mRNA encoded by the DNA identified by a polynucleotide sequence chosen from the group consisting of SEQ. ID NO: 1-122.

6. The transfected or transgenic plant of claim 5 wherein the plant is turfgrass.

7. The transfected or transgenic plant of claim 5 wherein the plant is fir tree.

8. A transfected or transgenic plant chosen from the group consisting of ornamental, horticultural, forestry, medicinal or Nicotiana sp. plants, exhibiting a dwarf phenotype made by the method comprising the steps of:

(a) providing a viral inoculum capable of infecting a plant comprising the DNA identified by a polynucleotide sequence chosen from the group of SEQ. ID NO: 1-122 or the mRNA encoded by the DNA identified by a polynucleotide sequence chosen from the group of SEQ. ID NO: 1-122;

- (b) applying said viral inoculum to a plant;
- whereby the plant is infected and the DNA or the mRNA is expressed in the plant.

9. The transfected or transgenic plant of claim 8 wherein the plant is turfgrass.

10. The transfected or transgenic plant of claim 8 wherein the plant is fir tree.

11. A method of producing multiple crops of the plant of claims 5-10 comprising the steps of:

- (a) planting a reproductive unit of the plant;
- (b) growing the planted reproductive unit under natural light conditions;
- (c) harvesting the plant; and

(d) repeating steps (a) through (c) at least once in the year. 12. A method of manufacturing a biopharmaceutical comprising:

- (a) providing a plant that expresses a biopharmaceutical in the plant;
- (b) providing a viral inoculum capable of infecting a plant comprising the DNA identified by a polynucleotide sequence chosen from the group of SEQ. ID NO: 1-122 or the mRNA encoded by the DNA identified by a polynucleotide sequence chosen from the group of SEQ. ID NO: 1-122;

(c) applying said viral inoculum to the plant;

whereby the plant is infected, exhibits a dwarf phenotype, and expresses the biopharmaceutical.

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